A STUDY OF HYPOLIPIDEMIC EFFECT OF LINSEED (*Linum usitatissmium* L.) IN THE DIET OF CARDIOVASCULAR PATIENTS

Thesis

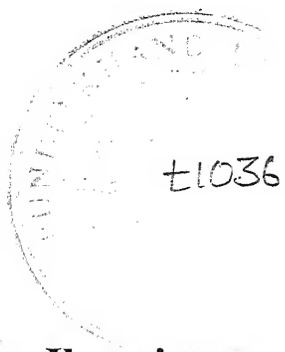
submitted for

Degree of Doctorate of Philosophy

in

Home Science (Foods and Nutrition)

to



Bundelkhand University, Jhansi

by

Mrs. Shalini Chakraborty

under the guidance of

Prof. Dheer Singh

Dedicated

to

my beloved

son

Neil

Acknowledgement

With limitless humility, I would like to take this unique opportunity to first thank "The Almighty" who bestowed me with the health and courage enough to go through the crucial juncture.

The memorable occasion provides me the privilege to express my sincere gratitude and thanks to the Hon'ble Vice Chancellor Prof.Ramesh Chandra of the Bundelkhand University, Jhansi.

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Place: JHANSI

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Certificate-I

This is to certify that work embodied in the thesis entitled "A Study of Hypolipidemic Effect of Linseed (Linum usitatissmium L.) in the Diet of Cardiovascular Patients" has been carried out by Mrs. Shalini Chakraborty under my supervision. She has fulfilled the requirements for the degree of Doctor of Philosophy in Home Science of Bundelkhand University, Jhansi, regarding the nature and prescribed period of investigational work. The work reported in this thesis embodies the work of the candidate herself and no part of this thesis has been submitted for any degree or diploma.

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Certificate-II

This is to certify that all the pathological experimental work embodied in the thesis entitled "A Study of Hypolipidemic Effect of Linseed (Linum usitatissmium L.) in the Diet of Cardiovascular Patients" has been carried out by Mrs. Shalini Chakraborty for fulfilling the requirements for the degree of Doctor of Philosophy in Home Science of Bundelkhand University, Jhansi, under my supervision in Raghvendra Hospital, Jhansi.

(R.R.Singh)

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List of abbreviations

%	percentage
µg	micro grams
ALA	alpha-linolenic acid
Anon.	Anonymous
AOAC	Association of official analytical chemists
CAD	Coronary Artery Disease
cal	calorie
CHD	Coronary heart disease
et al.	and others
gm	gram
HDL	High density lipo proteins
hr.	hour
IHD	Ischemic heart disease
Jr.	Junior
kg	kilogram
kcal	kilocalorie
l	litre
LDL	Low density lipo proteins
mg	milligram
ml	milliliter

N	Normality
nm	nano meters
O.D.	Optical density
^{o}C	Degree of celcius
PUFA	polyunsaturated fatty acid
tbs	tablespoon
TCA	trichloroacetate
t_{cal}	t-calculated value
TG	Triglycerides
tsp	tea spoon
VLDL	Very low density lipo proteins

INTRODUCTION

Chapter-I

INTRODUCTION

Food has been a wide range of naturally occurring substances for the human beings. The problem in the body system arise when the change in the food habits is too fast for the physiological adaptations. Disease results when the body is not able to compromise with these adaptations. Coronary heart disease is the leading cause of death in developed countries and the incidence is increasing in developing countries, including India **(Anon. 2000)**.

Coronary Heart Disease (CHD) also known as Coronary Artery Disease (CAD) or Ischemic Heart Disease (IHD), is the most deadly of cardiovascular disease involving the network of blood vessels surrounding and serving the heart. Coronary Heart Disease is now also considered as an important health problem in India. It is a part of epidemiological transition characterized by changing lifestyles and a probable genetic predisposition. The inter play of factors with regard to their existence, causality and attributable weightage needs to be understood in the context of management of an individual patient as well as strategic planning for control and prevention **(Yeolekar, M.E., 1998)**.

Coronary heart disease results from a lack of blood flow to the network of blood vessels surrounding the heart and serving heart muscle. The major

cause of CHD involves structural and compositional change in the innermost layer of the large arteries.

Researchers have identified several risk factors that put an individual at the risk of coronary heart disease. Some of these are controllable and some are uncontrollable. LDL cholesterol is the most sought for the risk factor of a CHD. Two LDL classes, with different risk have been identified. Phenotype A is indicated by very large LDL particles, which are not associated with risk of disease. By contrast, phenotype B is typified by small dense LDL particles that are triglyceride rich and cholesterol depleted and as predictive of CHD risk both in men and women (**Austin and Hokanson, 1994**).

Linseed are the hard tiny seeds, commonly known, as *alsi* is not only used as food source but also for other variety of purposes e.g. for the manufacture of high quality paints, varnishes, linoleum, oil, cloth and printer's ink (**Anon. 1962**). Linseed also known as flaxseed is one of the oilseed crop known since ancient times. The major linseed producing countries are United States, Canada, Germany and Russia. A considerable amount of the crop is also produced in other parts of the world including India.

Linseed has three components that have potentially very high health benefits. The three components that have been isolated are, - fibre, lignans,

and α –linolenic acid (a type of ω-3 fatty acids). Fibre is very useful in countering that problems related to the release of bowls. Research shows that α –linolenic acid and lignans are quite useful in prevention of cancer, curing kidney problems, and are very effective in treating cardiac ailments. Due to the presence of good amounts of ω-3 fatty acids, an essential fatty acid (α –linolenic acid), which comprises 57 per cent of the total fatty acid in Linseed, has a role in lowering the blood cholesterol level (**Kolodziejczykl *et al.*1995**). It can therefore be inferred that consumption of Linseed can reduce the risk of cardiovascular diseases and chances of CHD can be minimized.

Like other oilseed, linseed is also rich source of protein, fat and dietary fibres. Linseed also contains fair amount of minerals and carbohydrates. Linseed contains 6.5 per cent moisture, 37.1 per cent fat, 20.3 per cent protein, 4.8 per cent fibre and 2.4 per cent ash, (**Gopalan *et al.* 1995**). Linseed contains good amount of calcium and phosphorus, 170 and 370 mg/100g (**Aykroyd, 1966**).

It has been proved that linseed may be useful in the clinical management of a variety of conditions, especially those that involve the cardiovascular patients. However no substantial evidence is there where it is incorporated in the diet of the human beings. So the present study is planned to study the lipid lowering effect of linseed by incorporating it in the diet of

cardiovascular patients with the following **objectives** :

1. To study the physico-chemical analysis of linseed.
2. To study the health benefits of linseed.
3. To standardize incorporation of linseed to the traditional food formulation.
4. To conduct the sensory evaluation of the developed food formulation.
5. Supplementation of the diet of the cardiovascular patients with standardized amount of linseed incorporated selected traditional food formulation.
6. To study the effect of feeding the standardized amount of linseed on the blood lipid profile of the cardiovascular patients.

REVIEW OF LITERATURE

Chapter-II

REVIEW OF LITERATURE

Linseed commonly known as *alsi* is an oilseed crop belonging to family *Linaceae* and genus *Linum,* Linseed is an important oilseed crop of India and has a wide range of uses like,- for cooking purposes, for industrial purposes and for medicinal purposes also. In some parts of India its oil is used for the preparation of certain human and veterinary medicines and for preparation of cosmetics. Like other oilseeds, it possesses high nutritive value and contributes sizeable amount of not only fats but also proteins and fibres (**Deosthale and Longvah, 1988**).

Apart from nutritional significance, linseed has medicinal value also because of its higher dietary fibre constituents, linseed might reduces the coronary heart diseases which is a major health problem of western world but is now emerging as a major cause of death in Indian society also. Hyperlipidemia, an elevation of blood level of lipids, including cholesterol and triglycerides is associated with increased risk of Coronary Heart Diseases. Linseed being rich in polyunsaturated fatty acid (PUFA) and dietary fibre, might play an important role in lowering the blood cholesterol level. **Trowell (1972)** hypothesized that dietary fibre protect against hyperlipidemia and ischemic heart diseases.

2.1 Physical characteristics

Pryde (1983) studied some physical characteristics of linseed like seed shape, length, thickness and width. He found that linseed seed was flat and oval, with the pointed tip, length of linseed seed was 5 mm with thickness 2.5 mm and width 1.5 mm and the traditional linseed is shiny reddish brown in colour. **Yadava (1985)** also reported that linseed grown in India is usually brown in colour. According to the report on the marketing of linseed (in India 1939), weight of 100 large seeds may vary from 0.67 g to 0.89 g and weight of 100 small seeds may be around 0.5g.

2.2 Proximate Composition

Efforts have been made by a number of workers to ascertain the proximate composition of linseed (Table 2.1). **Marck and Rosenberg (1976)** reported the range of the various elements of proximate composition of linseed, such as moisture content, ash content, crude protein, crude fat, and crude fibre; 6.9 – 7.4 per cent, 3.9 - 4.8 per cent, 20.0 - 24.8 per cent, 37.8 - 43.2 per cent, 6.8 – 9.9 per cent, respectively. **Gopalan *et al.* (1995)** also conducted a detailed study and showed slightly lower level of proximate compositional constituents. **Punia (1998)** found that moisture, ash and protein are towards the lower side, while fat and fibre in the same range as reported by **Marck and**

Rosenberg (1976). Flax Council of Canada (1997) reported that linseed contains 4 per cent ash, 21 per cent crude protein, 41 per cent crude fat, and 28 per cent crude fibre on dry weight basis.

Table 2.1 Proximate composition of linseed

S.No	Worker	Year	Proximate composition, %				
			moisture content,	ash content	crude protein,	crude fat,	crude fibre,
1.	Marck and Rosenberg	1976	6.9 – 7.4	3.9 - 4.8	20.0 - 24.8	37.8 - 43.2	6.8 – 9.9
2.	Gopalan *et.al.*	1995	6.5	2.4	20.3	37.1	4.8
3.	Flax Council of Canada	1997	-	4	21	41	28
4.	Punia	1998	5.12 ± 0.26	3.5	20.56 ± 0.16	39.23 ± 0.37	8.40 ± 0.10

2.3 Available carbohydrates

Gopalan *et.al.* (1995) reported 28.5 per cent total carbohydrates content of linseed whereas **Punia (1998)** reported lower value of available carbohydrates i.e. 23.18 ± 0.28 per cent. **Madhusudhan and**

Singh (1983) reported that mucilages were the main carbohydrates present in linseed and it was to the extent of 7.45 per cent.

2.4 Mineral composition

Schultry and French (1978) revealed that linseed was a good source of potassium, phosphorous, calcium and magnesium. The calcium and phosphorous content of linseed was 170 mg/100g and 370 mg/100g respectively as reported by **Aykroyd (1966). Flax Council of Canada (1997)** analyzed the linseed for their mineral composition and reported that the linseed contains calcium – 236 mg/100g, potassium – 831 mg/100g, iron – 5 mg/100g , magnesium – 431 mg/100g, manganese – 3 mg/100 g, sodium – 27 mg/100g, copper – 1 mg/100g and zinc – 5 mg/100g. **Punia (1998)** reported that linseed contained 165 mg/100g calcium, 442 mg/100g phosphorous, 6.14 mg/100g manganese and 10.75 mg/100g iron.

2.5 Anti-nutritional factors

Whitney and Rolfes (1996) reported that linatine and phytic acid are substances that interfere with the absorption or metabolism of nutrients. **Flax Council of Canada (1997)** analysed that linseed contains 24.2 phytate μmol/Kg of linseed. **Punia (1998)** reported 152 $\pm$ 15.6 mg/100g of phytic acid in linseed.

2.6 Linseed and its health benefits

Consumers are turning to linseed for health benefits derived from its fibre, lignan and alpha –linolenic acid (ALA) content. Studies showed that consumption of linseed helps improve laxation and maintain blood glucose levels. Linseed appears to protect against certain types of cancer. Linseed reportedly reduces the blood cholesterol levels and hence the risks of cardio vascular diseases are also reduced. The linseed favourably affects immunity – the body's ability to defend itself successfully against foreign substances. Two components of linseed, alpha-linolenic acid (ALA) and lignans, affect immune cells. Recent research suggests that ALA and lignans in linseed modulate the immune response and may play a beneficial role in the clinical management of autoimmune diseases (**Blackburn, G.L. 1992**) and (**Parbtani *et al.* 1995**).

2.6.1 Linseed and laxation

Linseed, like cereals and legumes, has the potential to increase laxation because it provides dietary fibre which adsorbs water. This increases the intestinal bulk, which in turn increases laxation. This treatment has been especially found useful for the elderly as they do little physical exercise to ease their bowel movement easy (**Hamadeh *et al.* 1992**).

2.6.2 Linseed and cancer

Cancer is multi stage process influenced by environmental factors including diets. Studies indicate that dietary fats influence tumour development and /or growth. High intakes of ω-6 fatty acids, particularly linoleic acid are associated with tumour formation, where as high intakes of ω-3 fatty acids diminish tumour development (**Cave Jr., W. T. 1991**). Linseed contains two components that may help prevent or reduce the risk of certain types of cancers: lignans and alpha linolenic acid (ALA). ALA is an essential ω-3 fatty acid in the diet of the humans. Linseed is particularly a very rich source of alpha linolenic acid (**Raper *et al.* 1992**). Linseed is one of the richest sources of lignans (**Thompson,L.U. 1995**).

2.6.3 Linseed and cardio vascular diseases

Cardiovascular diseases are the result of atherosclerosis in which deposits of cholesterol and other lipids accumulate in and thicken the blood vessel wall, forming plaques. This process gradually restricts the blood flow. Thrombosis is the abrupt formation of clot initiated by blood platelet aggregation, causing a heart attack or stroke. Dietary fatty acids appear to be involved in both processes (**McDonald,B. E. 1993**). High blood cholesterol clearly contributes to cardiovascular diseases such as coronary heart diseases (CHD) and stroke, and diets high in fat

particularly saturated fat, are linked with high blood cholesterol levels. Diet that therapies to reduce blood cholesterol and the risk of CHD focuses mainly on reducing the total and saturated fat intake (**Expert Panel 1993, Cunnane,S.C. 1996**). **Virk Sanv *et al.* (2000)** found that dietary fibre has recently been recognised for reducing the risk of diabetes and heart disease. The implication is that it may have therapeutic benefit in prediabetic metabolic conditions. It was concluded that a diet rich in high viscosity dietary fibre improves glycemic control and lipid profile. **Trowell (1972)** reported that dietary fibre protects against hyperlipidemia and ischemic heart disease (IHD).

2.7 Therapeutic dosages

2.7.1 Stomach ailments

Fascicule, L. (1997) suggested usual doses of linseeds for constipation is 5 gm of whole, cracked or freshly crushed seeds soaked in water and taken with a glass full of liquids three times a day. **Turpila and Kivinen (1997)** reported that people received 6 to 24 gm per day of linseed for 6 months for constipation caused by irritable bowel syndrome.It was found out that consumption of soaked and strained whole linseed may help to soothe an upset stomach. For painful skin inflammation the recommended dosage of linseed is 30 to 50 gm of crushed powdered linseed applied externally **Fascicule, L. (1997).**

Turpila and Kivinen (1997) and **Arjamndi *et al.* (1998)** studied that linseed can help with chronic constipation and irritable bowel disease as it has high fibre content which also help in reduction of cholesterol. **Turpila and Kivinen (1997)** conducted a study on 55 people with chronic constipation caused by irritable bowel syndrome received either ground linseed or psyllium seeds daily for three months those taking linseed had significantly had fewer problems with constipation, abdominal pain and bloating than those taking psyllium.

2.7.2 Cardiovascular ailments

Arjamndi *et al.* (1998) conducted a study in which 38 elder women with high cholesterol ate bread containing either linseed or sunflower seeds for 6 weeks. Total cholesterol dropped with both regime but only those on the linseed had significantly lower LDL, the bad cholesterol. **Jenkins *et al.* (1999)** studied health aspects partially defatted linseed on 29 men and elder women with high cholesterol. They ate muffins with either defatted linseed or a wheat bran placebo for three weeks each. Those eating linseed showed significant decrease in both total and LDL cholesterol, compared to little change with wheat bran. **Harris, W.S. (1997)** suggested linseed oil as an alternative to fish oil for prevention of heart diseases. Linseed also causes 10 per cent reduction in total

cholesterol and 14 per cent reduction in LDL cholesterol **Turpila and Kivinen (1997).**

2.8 Linseed incorporation in food stuffs

Linseed because of its health benefits is being very commonly used in as a food ingredient in the western world as the population of health conscious people is increasing every day. Experimental work has shown little difference due to linseed variety in the physical and sensory qualities of food products in which they have been incorporated. **Malcolmson and Fyfe (1989)** examined the performance of adding linseed in three levels 10, 20, and 30 % to wheat flour. They reported that the linseed amount mattered most. Linseed bread had a nutty flavour. At 20 percent level the acceptance was good, more that 20 per cent resulted in the presence of an oily mouth feel after consumption of linseed-incorporated bread. Moreover at level 30 per cent the volume of the bread, and the crumb firmness, are also lost. In 1994 the annual consumption of linseed by the United States baking industry was estimated at 5000-7000 tonnes with the potential for an 8-10 fold increase. Milled linseed can be added to almost any baked product at levels of 6-8 % of the dry ingredients (**Carter,J. F. 1993**). Addition of linseed to the bread as an ingredient did not affect the odour intensity at

all as judged by a 9-member panel of sensory evaluators through a 128 days study (**Malcolmson *et al.*1996**).

To reap the rich health benefits of linseed; in addition to bakery products, linseed is also added to other food substances. Linseed is used as toppings to bolster the visual and sensory appeal of the food. A sprinkle of whole linseed makes an attractive alternate to poppy or sesame seed toppings. A sweet linseed snack agglomerated with fruit juices concentrate as an alternate to sesame seed snack bar (**Jenkins,D. J. A. 1995**). Stabilizers are added to the food formulations because of their ability to foam, or to emulsify fat, and /or bind water. These substances give a desirable mouth feel characteristics like smoothness or thickness and, when they gel, firmness. Both carbohydrates and protein extracts of linseed have the potential to perform the act of a food stabilizer (**Mazza *et al.* 1989 and Dev *et al.* 1989**). Linseed gum also finds wide use in the manufacture of breads, it is used as bread improver and to extend the shelf life of bread (**Garden-Robinson,J. 1994**).

2.9 Hypocholesterolemic effect of linseed

Linseed oil is rich in ω–3 fatty acids known to influence blood platelet aggregation, lower blood cholesterol concentration and prevent coronary heart diseases **Kolodziejczyl *et al.* (1995)**.

Hypocholesterolemic effect of linseed was observed in dogs fed on linseed. The dogs were fed without and with 0.625, 1.25, 2.5 and 5 per cent linseed. Linseed decreased the digestibility of the diets, particularly at low dosage. There was a decrease in mean serum cholesterol from 205-to 157 mg/100 ml in 5 per cent linseed group. Serum cholesterol was 166-164 mg/100 ml in control group. The total cholesterol in the liver fell with increasing linseed oil in the diet **(Kritchevsky *et al.* 1991)**. Similar study was also undertaken on rats. Weanling rats were fed on diets containing 0, 10, 20, 40 per cent linseed for 90 days. No differences were found in food intake or in body and organ weights but serum triacylglycerol, total cholesterol and low-density lipoprotein (LDL) cholesterol concentration were significantly lower in the rats fed on 20 and 40 per cent linseed diets than in the 0 per cent group. Lowering of LDL:HDL cholesterol ratio was also observed **(Ratnayake *et al.* 1992)**. **Bierenbaum *et al.* (1993)** reported that consumption of linseed decreased blood triglycerides slightly but not significantly. They also reported that serum total cholesterol and low-density lipoprotein (LDL) cholesterol values were reduced (7 and 11 per cent respectively) significantly but HDL – cholesterol did not change during linseed consumption by 15 hyperlipidemic subjects, fed on 15 g ground linseed daily.

The nutritional effect of linseed in humans was studied by **Cunnane *et al.* (1993)**. The report was that linseed lowered serum total cholesterol by 9 per cent and low-density lipoprotein cholesterol by 18 per cent when healthy women consumed 50 g ground linseed daily for 4 weeks. In the further study conducted it was revealed that consuming 50 g linseed daily for 4 weeks in young adults led to a significant increase in alpla-linolenate in adipose tissue and ω-3 polyunsaturates in plasma lipids. Plasma LDL cholesterol was also reduced upto 8% **(Cunnane *et al.* 1995)**.

Linseed was fed to growing pigs by **Barowicz *et al.* (1997)** to study its' hypocholesterolemic effect . Three groups of 12 pigs each were fed without and with 4 and 8 per cent linseed. They observed significant decrease in concentration of blood cholesterol (90.75 $\pm$ 4.76) and LDL fraction (42.38 $\pm$ 4.71) in 8 per cent linseed group than that of 4 per cent linseed group (120.44$\pm$ 5.23) and 61.62 $\pm$ 4.65) and no-linseed group (124.15 $\pm$ 9.54 and 67.55$\pm$ 10.30) mg/litre respectively. Hypocholesterolemic effect of feeding flax to pigs was attributed to high contents of unsaturated fatty (mainly linolenic) acids.

MATERIALS

AND

METHOD

Chapter-III

MATERIALS AND METHOD

The present investigations were conducted in the Department of Foods and Nutrition, Institute of Home Science, Bundelkhand University, Jhansi during 2002-2004 to study the hypolipidemic effect of linseed (*linum usitatissmium* L.) in the diet of cardiovascular patients. The materials, investigation methods, etc are reported in the following literature.

3.1 Procurement of Material

The Linseed *(Linum usitatissimum* L.*)* was procured from the local market. Linseed seeds were cleaned manually to get rid of dust and any other foreign materials.

3.2 Physical Characteristics

3.2.1 Colour

The linseed was observed for its colour and appearance.

3.2.2 Shape

The shape was recorded by vision as the seeds appeared.

3.2.3 Weight

One thousands seeds of linseed in triplicate were randomly selected and weighed in the electrical weighing balance.

3.2.4 Length and width

Fifty seeds of all of linseed were taken for length and width, which was determined with the help of vernier calliper.

3.2.5 Density

One thousand seeds were weighed in triplicate and were put in a graduated cylinder containing known amount of water and rise in water level was noted. Density was calculated by following formula:

$$\text{Density} = \frac{W(S)}{V(ml)}$$

Where W = Weight of 1000 seeds, gm

V = Rise in water level, ml

3.3 Preparation of Sample

Cleaned seeds of linseed were ground in stainless steel mixer grinder (**Plate 1**). These ground samples were stored in airtight plastic containers and kept in refrigerator till further analysis.

3.4 Chemical Analysis

3.4.1 Proximate composition

Moisture, Ash, Crude fat and Crude fibre contents were analyzed by using AOAC (1990) methods. Nitrogen was analysed by micro-kjeldhal method and multiplied by factor 6.25 for converting it into Crude Proteins.

3.4.2 Non- Protein Nitrogen

Non-protein nitrogen was determined by the method of Becker *et. al.* (1940).

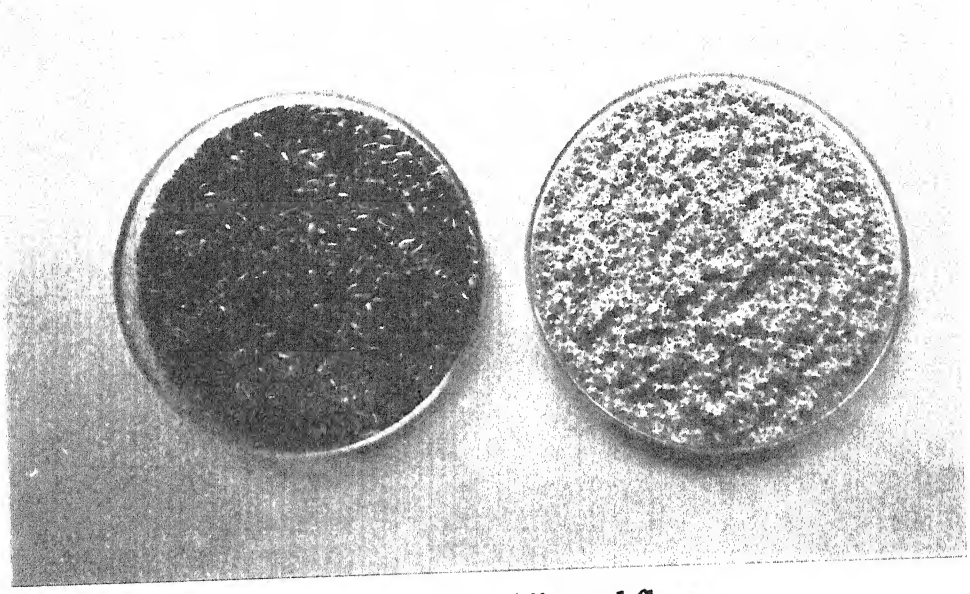

Plate 1 Linseed and linseed flour

Reagents:

Trichloroacetic acid (10%) solution: Ten gram of tricholoroacetic acid was dissolved in 100 ml of distilled water.

Procedure:

Five hundred milligram sample in triplicate was extracted with 10 ml ice cold 10% TCA and centrifuged at 10,000 rpm for 5 min. The precipitates were washed twice with 10% TCA, supernatent was pooled and volume was made to 50 ml with 10% TCA. 25 ml of aliquot was taken and digested. Nitrogen content was analyzed by micro- kjeldhal method (AOAC, 1990).

$$\text{Non} - \text{prote in nitrogen}(\%) = \frac{\text{Volume of HCl used x N of HCl x 14 x Volume of digest x 1000}}{\text{Aliquot of digest taken x Weight of sample}}$$

3.4.3 True protein

True protein was calculated by the following formula:

True protein = (Crude protein nitrogen – Non-protein nitrogen) X 6.25

3.4.4 Energy

Energy in sample was determined by Chromic oxide method of **O'Sheer and Mayure (1962).**

Reagents:

1.5 N Potassium dichromate solution: Dissolved 73.54 g of potassium dichromate (AR) in 100 ml warm distilled water and make the volume to one litre.

0.15 N sodium thiosulphate solution: Dissolved 37.23 g of sodium thiosulphate in 100 ml of warm distilled water and volume was made to one litre

Potassium iodide sodium bicarbonate solution : Hundred gram of potassium iodide (AR) and 32 g of sodium bicarbonate were dissolved in distilled water and diluted to 500 ml. These were prepared fresh.

Concentrated Sulphuric acid

Procedure: -

Hundred twenty-five mg sample was oxidized with 20 ml 1.5 N potassium dichromate solution and 40 ml of concentrated sulphuric acid for 90 minutes. The total volume was made to 250 ml. To a 25 ml aliquot, 10 ml of potassium iodide sodium bicarbonate solution was added in dark, avoiding exposure to light for 25 minutes. The contents were diluted with 50 ml of water and iodine liberated was titrated against 0.15 N sodium thiosulphate using strach as indicator. End point was light green colour. The duplicate blanks were also run for each set. The amount of 1.5 N $K_2Cr_2O_7$ used for oxidising the samples

was calculated by subtracting the above reading from the blank. The energy values were calculated by following equation.

$$\text{Kcal/100gsample} = \frac{\text{ml of 1.5N } K_2Cr_2O_7 \text{ used for oxidising 1g of sample} \times 100}{\text{oxidizingcoeficient}}$$

Oxidising coefficient = $23.39 - 0.069P + 0.00026P^2$

Where, P= true protein content of sample.

3.4.5 Available Carbohydrates

Total sugars other than starch were extracted according to procedure by **Cerning and Guilbot (1973).**

Reagent: -

Ethanol 80%

Procedure: -

Extraction

Twenty five ml ethanol (80%) was added to a 500mg sample in a round bottom flask. The flask was connected to condenser and kept on a heating mantel for 30 minutes with occasional stirring. The extract was cooled, centrifuge at 8000 rpm for 15 min. and the supernatant was collected. The above procedure was repeated twice, each time extracting the residue in 25 ml 80% ethanol. The combined extract

was evaporated to dryness in a beaker on a boiling water bath. The residue was dissolved in distilled water and made to 50ml.

3.4.5.1 Total soluble sugars

Total sugars were estimated by the method of **Yemn and Willis (1954).**

Regents

Standard sugar solution: Dissolved 25 mg glucose in water and made to 100 ml to 1.0 ml of this solution was used.

Anthrone reagent (0.2% anthrone in 70% sulphuric acid). This reagent was prepared fresh daily and allowed to stand for 30-40 min. before use.

Procedure

Estimation

Freshly prepared 10ml anthrone reagent was pipetted into a test tube (150 x 25 mm), chilled and kept in ice-cold water. One ml of the sugar extract was taken and diluted to 10 ml with water. Out of the diluted extract one ml was taken and was layered on the anthrone reagent. After cooling for 3-5 min. the contents were thoroughly mixed while still emerged in ice-cold water. The contents in the tube were heated vigorously in boiling water. The contents in the tube were heated vigorously in boiling water for 10 minutes and then

immediately cooled in cold water. The absorbance was then at 256 nm against a suitable blank.

3.4.5.2 Reducing sugars

Reducing sugars were estimated by Somogy's modified method (Nelson, 1942, Somogy, 1945).

Reagents

Copper reagents A: Dissolved 25g anhydrous sodium carbonate, 25g potassium sodium tartarate, 20g sodium carbonate and 200g anhydrous sodium sulphate in about 800ml distilled water and diluted to one litre.

Copper regents B: Dissolved 15g $CuSO_4$ in 100ml distilled water containing two drops of HCl.

Arsenomolybdate reagent: Dissolved 25 g ammonium molybdate in 450ml distilled water and 21 ml concentrated sulphuric acid with string, later 3g sodium hydrogen arsenate in 25ml distilled water was also mixed by stirring. The solution was kept in an incubator at 37° C for 24 hours use. The regents were stored in glass stoppered brown bottle in a refrigerator.

Mixed copper regent: Copper regents A and B were mixed in the ratio of 25:1 (V/V) before use.

Standard sugar solution: - Dissolve 25 mg glucose and made to 100 ml with water. This contained 250-µg glucose/ml.

Procedure :

One ml of test extract obtained in 3.4.5 was taken in a blood sugar tube graduated at 25ml. One ml mixed copper water regents was added and then heated for 20 min. In boiling water bath. To this one ml arsenomolybdate regent was added, thoroughly mixed and the contents diluted to 25ml. A stable blue appeared quickly which was read at 520 nm against the suitable blank. The amount sugar was then determined by referring it to the glucose standard curve.

3.4.5.3 Non– reducing sugars

The amount of non-reducing sugar was calculated by subtracting reducing sugars from the total invert sugars and multiplying the difference by a factor of 0.95.

3.4.5.4 Starch

Starch was estimated by the method of **Clegg (1956)**.

Reagent

Ethanol 80%

Standard sugar solution: -

Dissolved 25mg glucose in water and made to 100ml (this solution containing 250 µg glucose/ml) for obtaining the standard curve, 0.1ml to 1.0 ml of this solution was used.

Anthrone reagent (2% anthrone in 70% sulphuric acid) This reagent was prepared fresh daily and allowed to stand for 30-40 min. before use.

52% per chloric acid.

Extraction :-

Twenty-five ml ethanol (80%) was added to 500 mg sample in round bottom flask. The flask was connected to condenser and kept on a heating mantel for 30 minutes with occasional stirring. The extract was cooled, centrifuged at 8000 rpm for 15 min. and the supernatent was discarded. The above procedure was repeated twice each time extracting the residues in 25 ml of 80 per cent ethanol. This sugar free pellet was used for the estimation of starch.

Added 5ml of water to the aforsaid sugar free pellet in test material and while stirring added 8.5ml of 52% perchloric acid. Stirred the contents vigorously for five minutes and then occasionally for next 15 min. Added 20ml water and centrifuged. Collected the supernatant in 100ml volumetric flask. Added 5ml water to the

residue and repeated the extraction with 52% perchloric acid, stirring occasionally for next 30min. washed the content of the tube into a volumetric flask. The rest extract was made to 100ml with water. It was then filtered, discarding first 5ml of filtrate. A suitable aliquot of extract was used for glucose estimation.

Estimation: -

Freshly prepared 10ml anthrone reagent was pipetted into a test tube. Chilled and kept in ice cold water. One ml of the sugar extract was taken and diluted to 10ml with water. Out of the diluted extract one ml was taken and layered on the anthrone regent. After cooling for 3-5 min. the contents were thoroughly mixed while still emerged in ice cold water. The contents in the tube were heated vigorously in a boiling water both for 10 minutes .and then immediately cooled in cold water. The absorbency was then read at 526 nm against a suitable blank.

Starch was calculated using the following formula:

Starch = Glucose x 0.9

3.4.6 Unavailable Carbohydrates

3.4.6.1 Neutral detergent fibre

Reagents:

Neutral detergent solution: -

Distilled water	1 litre
Sodium launyl sulphate	30 g
Diasodium ethylene diamino tetra-acetate (EDTA)	18.61 g
Sodium borate decahydrate	6.18g
Disodium hydrogen phosphate	5g
2-Ethoxy ethanol	10.0 ml

EDTA and sodium borate decahydrate were put in large beaker. Some distilled water was added and heated until these are dissolved, then Sodium launyl Sulphate and 2- ethoxy ethanol were added. In a separate beaker disodium hydrogen phosphate was dissolved in distilled water by heating. Both the solutions were mixed and volume was made to one liter. Adjusted pH between 6.9 and 7.1.

Decahydronapthalene - (AR)

Acetone - (AR)

Sodium sulphite, anhydrous - (AR)

Procedure: -

One-gram air-dried sample (in triplicate) was weighed into a beaker of the refluxing apparatus. One hundred ml neutral detergent solution, 2ml decahydronapthalein and 0.5 g , sodium sulphite were added to it. Then it was heated to boiling. Heat was reduced as boiling began, to avoid foaming and allowed to reflux for 60 minutes. It was filtered through a weighed glass crucible with the minimum of hot water. Liquid was filtrated and washing procedure was repeated. Then the washing was done with acetone in the same manner and dried. The crucible was dried in hot air oven at 100° C for 8 hours and weighed after cooling. NDF was calculated using the following formula :

$$\text{NDF}\,\% = \frac{\{(\text{weight of crucible} + \text{fibre content}) - \text{weight of crucible}\}}{\text{weight of sample}} \times 100$$

3.4.6.2 Acid detergent fibre

Reagent: -

Acid detergent solution

1N Sulphuric acid solution

Cetyl trimethyl ammonium bromide (CTAB): 20.00g. Added twenty g of CTAB to 1N sulphuric acid and made the volume to one litre.

Decahydronapthalene

Acetone

N-hexane

Procedure: -

One-gram air dried sample (in Triplicate) was weighed in a beaker of the refluxing apparatus. 100ml of acid detergent solution and 2ml of decahydronapthalein were added to it. The mixture was heated to boiling and refluxed for 60 min. Filtered it through a weighed glass crucible on filter manifold. Rinsed the sample into crucible with minimum of hot water (90 to 100°C). Finally the residue was washed twice with acetone in the same manner till colourless. All lumps were broken so that solvent came into contact with all particles of the fibre. The acid detergent fibre was dried at 100°C for 8 hours in hot air oven and weighed.

Calculations: -

$$\text{ADF}\,\% = \frac{\{(\text{weight of crucible} + \text{fibre content}) - \text{weight of crucible}\}}{\text{weight of sample}} \times 100$$

3.4.6.3 Hemi cellulose

Hemicellulose was determined as the difference between NDF and ADF.

Hemicellulose = NDF-ADF

3.4.6.4 Cellulose

The difference between ADF and lignin gave cellulose.

Cellulose = ADF-lignin

3.4.6.5 Lignin

Lignin was determined by the method of **Van Soest (1967)**

Reagent: -

H_2SO_4,72% by weight

Preparation: -

Estimation of Lignin requires preparation of ADF first. Took 417ml distilled water in a volumetric flask and added 583 ml pure H_2SO_4 slowly with occasional stirring.

Procedure: -

Prepared the ADF, Filled the crucible (sintered) containing ADF with72% H_2SO_4,(15°C) and stirred with glass rod to smooth the paste and break the lumps. Glass rod was left in the crucible, refilled with 72% H_2 SO_4 and stirred at hourly intervals so that the acid may drain away. The crucible was kept at 20-23°C.

After three hours, filtered as much as possible. Washed the content with hot water until it was free from acid. Rinsed and removed the glass rod. Dried the crucible at 100°C for 8 hour overnight and weighed. Kept the crucible in muffle furnace at 500-550°C for 3 hours. Cooled and weighed.

Calculations: -

$$\text{Lignin \%} = \frac{\{(\text{weight of crucible} + \text{lignin}) - (\text{weight of crucible} + \text{weight of ash})\}}{\text{weight of sample}} \times 100$$

3.4.7 Minerals: -

Digestion

One-gram ground sample was taken in a 150 ml conical flask. To this 25-30 ml of diacid mixture (HNO_3, $HClO_4$ in 5:1 V/V) was added and kept overnight. Digestion was done next day by heating till clear white precipitate settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrate was made to 50ml with double distilled water and used for determination of magnesium, zinc, copper, iron , phosphorous and calcium with the help of atomic absorption spectrophotopmeter, model 3100, Perkin Elmer. Sodium and potassium were determined with the help of flame photometer, Mediflame, 127.

3.4.7.1 Phosphorus

Phosphorus was determined calorimetrically by the method of **Chen *et al* (1956).**

Reagents

Ascorbic acid solution (10%)

Ammonium molybdate Solution (2.5%)

Regents C: Mixed 6N H_2SO_4, water, 2.5% ammonium molybdate solution and 10% ascorbic acid solution in the ratio of 1:2:1 V/V, respectively. This regent was prepared fresh every day.

Standard phosphorus solution: Dissolved 0.351g pure and dry anhydrous mono potassium dihydrogen orthophosphate in a few ml of water and added 10ml of 10N H_2SO_4, solution. The volume was made to one litre with water. This stock solution contained 80ug P/ml. Diluted stock solution to one litre which served as working standard solution. It contained 2ug P/ml. Two or three drops of chloroform were added for preserving this solution.

Procedure:

One ml of the mineral extract from 3.4.9.1 was diluted to 10ml. From this, one ml was pipetted in a test tube and made volume to 4ml with water. Added 4ml regents C and mixed well. Incubated the contents at 37° C in a water bath for 90 min. Removed and allowed to cool to room temperature and read absorbence at 820nm against a suitable blank. Standard curve was plotted using 1 to 8ml of standard phosphate 0.2 O.D. correspond to 2µg phosphorus.

3.4.8 Phytic acid: -

Phytic acid was determined by the method of **Davis and Reid (1979).**

Reagents: -

0.5 N HNO_3: 15.96 ml of 69.5 % HNO_3 (AR grade Sp. Gravity 1.4) was diluted in water and made the volume to 500ml.

Ferric ammonium sulphate solution: -

Two hundred sixteen milligram ferric ammonium sulphate was dissolved in water and made to 500ml.

Ammonium thiocynate 10% solution: -

Ten-gram ammonium thiocynate was dissolved in water and made to 100 ml.

Isoamyl alcohol: -

Sodium phytate standard:

30.54 mg sodium phytate (5.5%, H_2O, 97% purity and containing 12Na/mol.) was dissolved in 100 ml 0.5 N nitric acid which gave a solution containing 200µg phytic acid per ml.

Standard curve: -

Different concentrations i.e. 0.2 to 1.0ml of standard sodium phytate containing 40-200µg phytic acid were taken and made to 1.4ml with water.

Procedure: -

Extraction:

One-gram sample was extract with 25ml of 0.5 N nitric acid for 3 hours with continuous shaking in a shaker. After proper shaking, it was filtered through Whatman No. 1 filter paper.

Estimation: -

Pipetted 0.5 to 1ml filtrate in a test tube and diluted with distilled water to a final volume of 1.4 ml. One ml ferric ammonium sulphate solution was added to it. The tube was after proper shaking placed in boiling water both for 20 minutes. After cooling down to room temperature under running tap water 5ml isomyl alcohol was added. The tube were shaken well and then centrifuged at 3000 rpm for 10 minutes.

Finally, the colour intensity was read at 465 nm against amyl alcohol blank exactly after 15 min. of addition of ammonium thiocynate . The extraction at 465 nm is amyl alcohol layer was inversely proportional to phytate concentration.

Calculations:

$$\text{Phytic acid (mg/100g)} = \frac{\text{reading of graph x ml of volume made}}{\text{weight of sample taken x ml of aliquot taken}}$$

3.5 Health benefits of linseed

The cultivation of linseed (*Linum usitatissmium* L.) for its fibre and nutritive value is an ancient practice since ancient times. Many of the health benefits of linseed can be gained by consuming freshly ground linseed (**Cunnane *et al.* 1993**). Linseed is rich in ω-3 fatty acids. Linseed oil contains the highest amount of ω-3 fatty acids, 55%. Increasing the consumption of linseed will lower the unhealthy ω-6: ω-3 ratio. This will be a huge bonus for the cardiovascular patients

3.6 Incorporation of linseed in traditional food product

The linseed was fed to the subjects by means of traditional food products. This method of administering the dosage of linseed was chosen so that the subject may consume it as and when the diet is consumed.

3.6.1 Preparation of sample

The linseed procured from the local market was cleaned of all the foreign matter and then ground in a mixer grinder to form a consistent powdered mass, can be called as linseed flour. This linseed flour was added to different preparation as an ingredient in predetermined proportions.

3.6.2 Formulation of products

Different traditional food formulations viz *ladoo, roti, chutney, mathri*, pan-cake (*cheela*), and *dhokla* were prepared with linseed incorporated at three different levels. The method of preparation was as per the standard recepies. The linseed was added in three levels, - 5, 7, and 9 per cent of the dry weight of the major ingredients.

Table 3.1 Ingredients used for *ladoo*

Ingredients	Amount (gm)			
	A	B	C	D
Wheat Flour	100	95	93	91
Linseed Flour	-	5	7	9
Sugar	20	20	20	20

A=control, B=5% linseed, C=7% linseed, D=9% linseed.

Table 3.2 Ingredients used for *roti*

Ingredients	Amount (gm)			
	A	B	C	D
Wheat Flour	100	95	93	91
Linseed Flour	-	5	7	9

A=control, B=5% linseed, C=7% linseed, D=9% linseed.

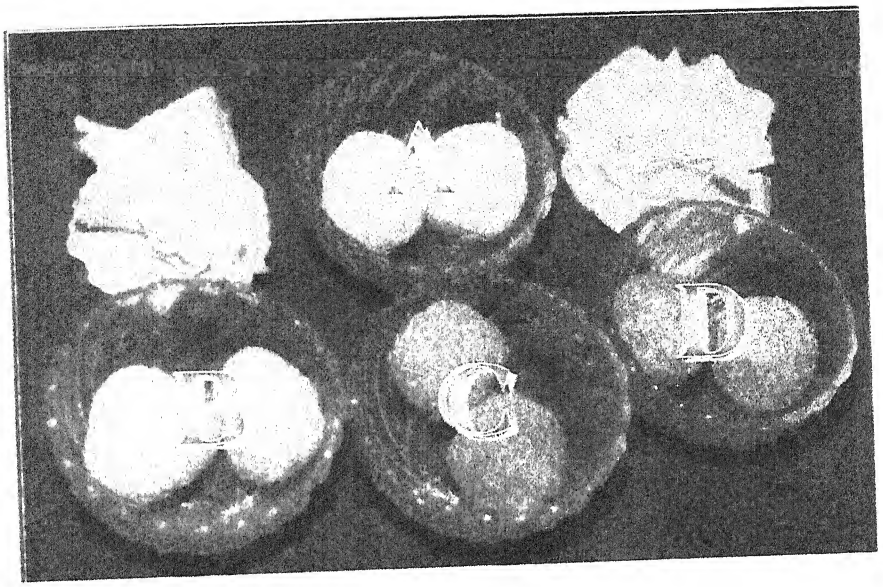

Plate 2 Linseed incorporated *ladoo*

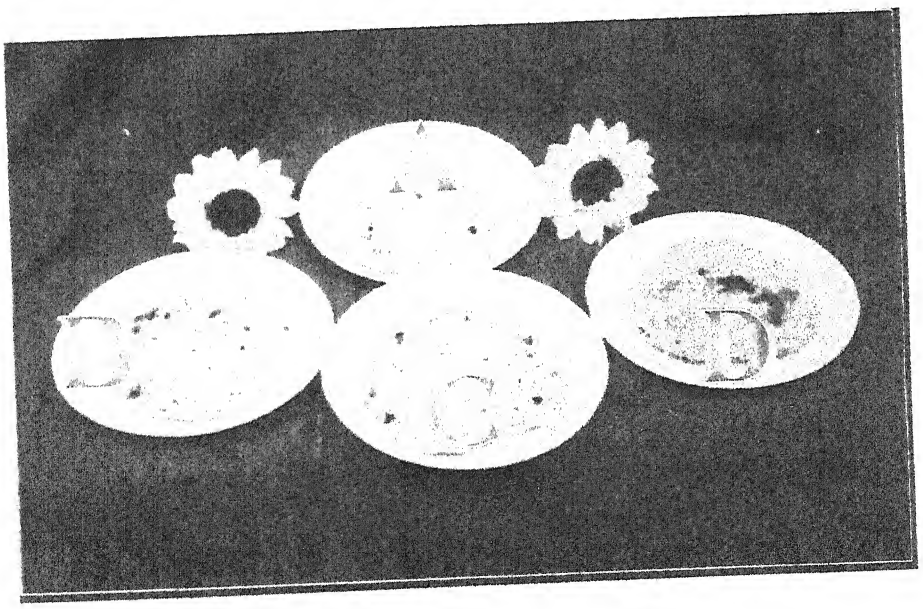

Plate 3 Linseed incorporated *roti*

Table 3.3 Ingredients used for *mathri*

Ingredients	Amount (gm)			
	A	B	C	D
Wheat Flour	100	95	93	91
Linseed Flour	-	5	7	9
Shortening	30	30	30	30
Salt	To taste			
Oil	For frying			
	A pinch			

A=control, B=5% linseed, C=7% linseed, D=9% linseed.

Table 3.4 Ingredients used for *Chutney*

Ingredients	Amount (gm)			
	A	B	C	D
Coriander leaves	100	95	93	91
Mango Powder	30	30	30	30
Linseed Flour	-	5	7	9
Jaggery	20	20	20	20
Green Chili	2	2	2	2
Salt	To taste			

A=control, B=5% linseed, C=7% linseed, D=9% linseed

Plate 4 Linseed incorporated *mathri*

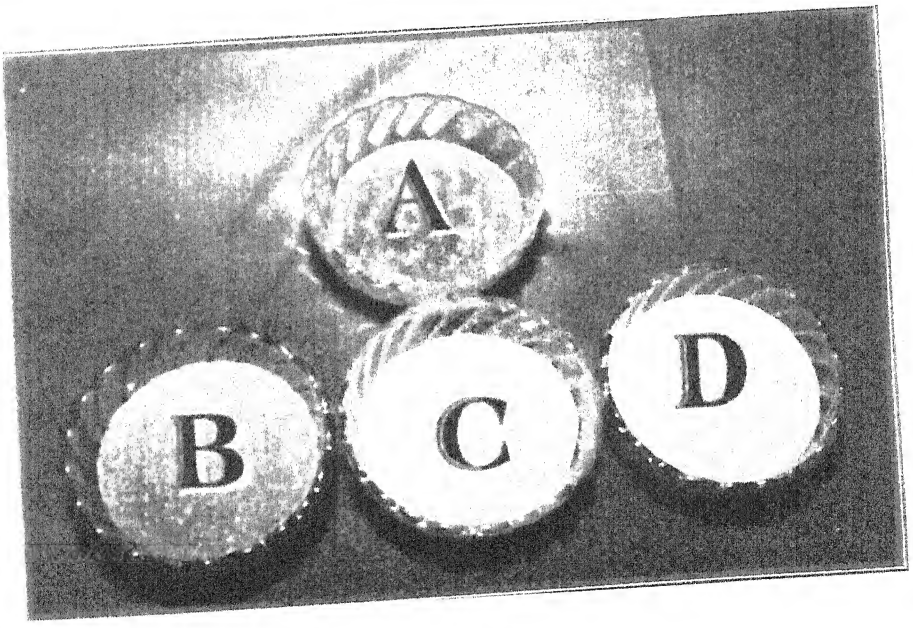

Plate 5 Linseed incorporated *chutney*

Table 3.5 Ingredients used for *Dhokla*

Ingredients	Amount (gm)			
	A	B	C	D
Bengal Gram Flour	100	95	93	91
Linseed Flour	_	5	7	9
Sugar	5	5	5	5
Green Chilli	2	2	2	2
Mustard Seeds	A pinch			
Salt	To taste			

A=control, B=5% linseed, C=7% linseed, D=9% linseed.

Table 3.6 Ingredients used for *Pancake(Cheela)*

Ingredients	Amount (gm)			
	A	B	C	D
Bengal Gram Flour	100	95	93	91
Linseed Flour	_	5	7	9
Green Chilli	2	2	2	2
Dry Mango Powder	2	2	2	2
Salt	To taste			
Oil	For frying			

A=control, B=5% linseed, C=7% linseed, D=9% linseed.

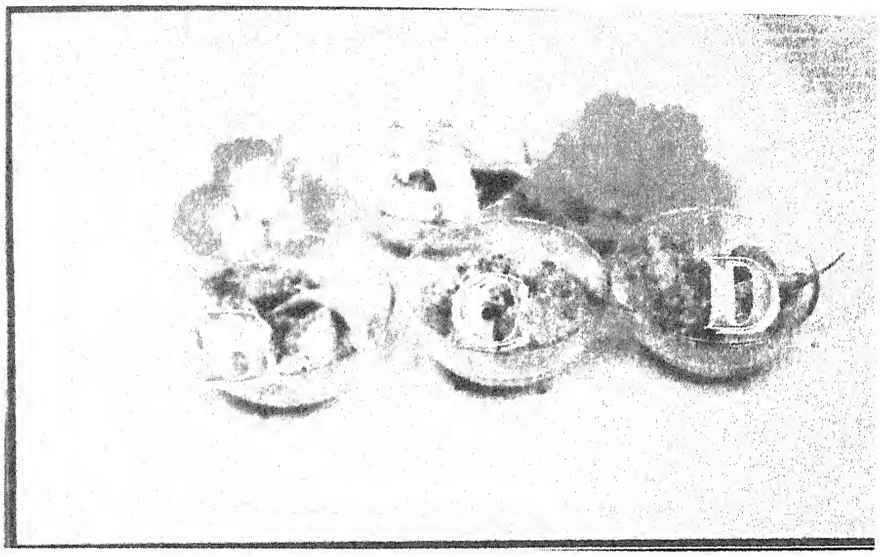

Plate 6 Linseed incorporated *dhokla*

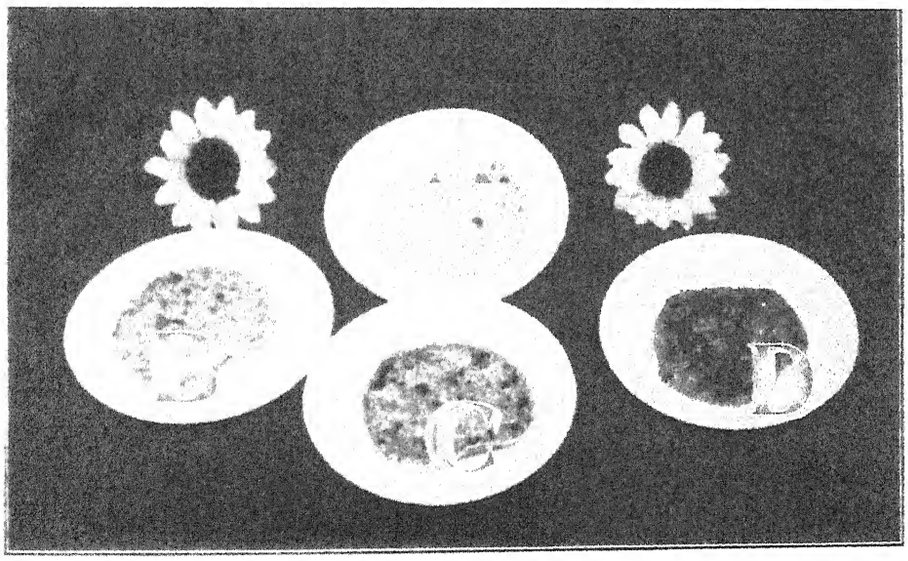

Plate 7 Linseed incorporated pancake*(cheela)*

3.6.3 Nutritional composition of food formulations

Different nutritional attributes viz. calorie, protein, fat, fibre mineral, were calculated for the all-traditional food formulations. The calculations were carried out by using the nutritional values given by **Gopalan *et al.* (1995).**

3.7 Sensory evaluation

When the quality of the food is to be assessed by means of human sensory organs, the evaluation is said to be sensory. Sensory quality is a combination of different senses of perception, appearance , flavour, and mouth feel decide the acceptance of the food.

3.7.1 Sensory characteristics of food

This include certain parameters that would help assessing the quality of food. These parameters are,- appearance, colour, flavour, psychological factors. Appearance is as a result of the surface characteristics of the food. As appearance vary for cooked and uncooked food so would the acceptability. The flavour of the food has three components- odour, taste, and composite of these sensations called mouth feel. Odour is the property by the means of which molecules of volatile compounds come in contact with the receptors of the olfactory organs. Taste is registration made by the tongue as and when some food substance is consumed. It can be categorized into, salty- taste generated by the ions present in the

salt, sweet- taste generated by the organic compounds present in the food substance, sour- taste generated by the hydrogen ions present in the food products. Mouth feel can be summarized as texture, consistency, and the burning sensation that is elicited by the food product upon their touch to the mouth.

3.7.2 Conducting sensory tests

A panel of trained judges conducted the tests and the food formulations were evaluated. A panel was required because of the inherent limitation of an individual to judge and a give unbiased report regarding the evaluating attributes of the food substance. It was ensured that the panel of judges were fresh before the start of the tests. The number of panel member was 10 for the present study. The samples that was presented for the tests was from a homogeneous lot. All the samples were evaluated in the same ambience. The samples were coded to conceal the identity. An evaluation card is a very important element for conducting a successful sensory tests. The score card was so designed that all the key characteristics of the product was evaluated. Hedonic rating test **(fig 3.1)** was used to measure the consumer acceptability of the products on the scale of 9 points, ranging from 'extremely liked' (scoring 9 points) to 'disliked extremely' (scoring 0 points). Further analysis was made based on the ratings as given by the panel members.

Hedonic Rating Test

Name:---------- Date----------

Product:----------

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feelings about the sample. Please give reason for this attitude. Remember you are only one who can tell what you like. An honest expression of your personal feeling will help us.

	code	code	code
Like extremely	----------	----------	----------
Like very much	----------	----------	----------
Like moderately	----------	----------	----------
Like slightly	----------	----------	----------
Neither like dislike	----------	----------	----------
Dislike slightly	----------	----------	----------
Dislike moderately	----------	----------	----------
Dislike very much	----------	----------	----------
Dislike extremely	----------	----------	----------

Reason

Figure 3.1 A 9 point Hedonic Scale

3.8 Selection of level of addition of linseed

The linseed had to be incorporated in the traditional food products viz. *ladoo*, *roti*, *dhokla*, pan cake (*cheela*), *chutney* and *mathri*, so that the effect of feeding linseed to the subject can be ascertained. The range of level of linseed to be added was retrieved form the review work carried out during the course of the study. The linseed was added in three levels, - 5, 7, and 9 per cent of the dry weight of the major ingredients used for preparing all the six traditional food formulations.

One set of the six traditional food formulations was prepared with no linseed or 0% linseed, called control. To make sure that addition of linseed did not cast any displeasing characteristic to the traditional food formulation a sensory evaluation test was carried out to ascertain the overall acceptability of the linseed incorporated traditional food formulations. The food products prepared were then served to a panel of 10 sensory evaluators. As per the verdict of the panelists the sensory scores were recorded. The average score of the 10 sensory evaluators for every particular level (5, 7, and 9 per cent) was compared with the average sensory score of the control (0% linseed). Paired t-test statistics was used to confirm whether the incorporation of linseed had significantly affected the overall acceptability of the food formulations.

3.9 Selection of a food formulation

There were six different food formulations in which linseed was incorporated. The food products prepared were then served to a panel of 10 sensory evaluators for selecting a particular product which was to be administered to the hyperlipidemic subjects. As per the verdict of the panelists the sensory scores were recorded. The average score of the 10 sensory evaluators for every particular traditional food formulation with selected level of linseed incorporation was compared with the average sensory score of the traditional food formulation with no linseed (control, 0% linseed). The results were then scaled on a nine point Hedonic Rating Scale and the over all acceptability of the products were understood. On the basis of the results of this test one of the developed food product was chosen. This chosen product was then administered to the hyperlipidemic subjects. Paired t-test statistics was used to confirm whether there was any significant difference in the over all acceptability of the traditional food formulation with selected level of linseed incorporation and the overall acceptability of the traditional food formulation with no linseed (control, 0% linseed).

3.10 Hyperlipidemic subjects

Hyperlipidemia is state when there is a rise in the level of blood lipids content. Fifty hyperlipidemic subjects randomly selected for the study.

The status of the patients being hyperlipidemic was judged on the basis of total serum blood cholesterol, low-density lipoprotein (LDL) cholesterol, serum triglycerides, very low-density lipoprotein (VLDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. While total serum blood cholesterol, high-density lipoprotein (HDL) cholesterol, and serum triglycerides were estimated using standard analytical techniques in pathological laboratories, the low-density lipoprotein (LDL) and (VLDL) cholesterol, and was estimated by using the **Friedewald (1972)** equation. At the start of the study the blood lipids content of the hyperlipidemic subjects were measured. These subjects were then allowed to maintain their usual pattern of diet for a period of six weeks. At the completion of six weeks, the blood lipids content of the hyperlipidemic subjects were again measured. The change, if any was registered. These same subjects were then served the selected food formulation with linseed incorporated at the selected level for the same period (six weeks). The change in the blood lipids content as effected by the consumption of linseed incorporated food formulation was registered. Percentage change in the blood lipids content was recorded for the first six weeks , when subjects maintained their usual diet pattern, and for the next six weeks when the subjects consumed the selected food formulation with linseed incorporated at the selected level.

Paired t-test statistics was used to confirm whether there was any significant difference in the blood lipids content after the selected food formulation with linseed incorporated at the selected level was consumed by the subjects.

RESULTS

AND

DISCUSSION

Chapter-IV

RESULTS AND DISCUSSION

Experiments were conducted to answer the objectives of the research work. The physical and chemical analysis of linseed was conducted in detail. Six traditional linseed incorporated food formulations were prepared. A particular linseed incorporated food formulation was selected on the basis of its consumer acceptability. The selected food formulation was then fed to the subjects to see the hypocholesterolemic effect of linseed. The following chapter will present the result along with suitable discussion of all the experiments that have been conducted during the course of study

4.1 Physical characteristics

One thousands seeds of linseed in triplicate were randomly selected and weighed in an electrical weighing balance. The mean of the three weights was considered for the final reading, and it was found to be 6.05gm.

The colour of linseed used for the study is as shown in plate 1. The colour, as it can be seen from the plate was dark brown with shiny surface texture.

The seeds were oblong in shape with flat surface and pointed tips. Fifty seeds of linseed were randomly taken for length and width, which was

determined with the help of vernier calipers. The average length and width of seeds was 5.45 mm and 1.45 mm, respectively.

Density is defined as weight per unit volume. The density was determined by volume displacement method, and was found to be 720 kg/m^3.

Table 4.1 Physical characteristics linseed (*Linum usitatissimum* L.)

S.No.	Physical characteristics	Result
1	Colour	Shiny dark brown
2	Shape	Flat oval with slight pointed tip
3	Weight (g)*	6.05
4	Length (mm)	5.45
5	Width (mm)	1.45
6	Density**	0.690

* of 1000 seeds

** $\frac{\text{weight (1000 seeds)}}{\text{volume (ml)}}$

4.2 Prepared sample

The linseed used for the study was made into linseed flour by grinding it in a mixer grinder. The particle size of the flour was reduced to a size such that the flour could pass through a 40 mesh sieve.

4.3 Chemical analysis

4.3.1 Proximate composition

Proximate composition gives useful information about the nutritional and biochemical quality of food. Nutritive value of any food and food product depends mainly on their proximate composition, which includes determination of percentage of moisture, ash, crude fat, crude protein and crude fibre.

4.3.1.1 Moisture

Determination of moisture content is one of the most important widely used analytical measurement. Moisture content is an index of stability and quality and also is a measure of yield and quantity of food solids. Moisture content of linseed was 6.25%.

4.3.1.2 Ash

Ash content of foods, in general, is defined as the inorganic residues left over after incineration. The estimation of ash in foods also reveals the mineral constituents, which plays an important role in human nutrition. The ash obtained is not necessarily of exactly the same composition as there may be losses due to volatilization or some interaction in between the constituents. The ash content of linseed used for the study was found to be 4.17 % of the dry weight.

4.3.1.3 Crude fat

The ether extract or crude fat content of a food represents the true fat (triglycerides) and other materials such as phospholipids, sterols, essential oils, fat-soluble pigments, waxes, carotenoids, chlorophyll and other pigments extractable with ether. The linseed used had a fat percent of 42.65.

4.3.1.4 Crude protein

Proteins are indispensable components of living matter, chemically they are nitrogenous compounds. In foods, proteins supply the essential amino acids, which are necessary to sustain life. protein content of linseed was 19.06 per cent.

Table 4.2 Proximate composition of linseed (*Linum usitatissimum L.*)

S.No.	Proximate composition	Result (%)
1	Moisture	6.25
2	Ash	4.17
3	Crude fat	42.65
4	Crude Protein	19.06
5	Crude Fibre	8.05

4.3.1.5 Crude fibre

Plant foods apart from containing available carbohydrates and starch, which are easily digested and absorbed by the small intestine, also

contains fibrous or viscous polysaccharides which gives plants, their structure and form but are not digested in the human system, are known as fibre content of food. On carrying out the required procedural test for the determination of crude fibre, it was estimated to be 8.05 per cent.

4.3.2 Available carbohydrates

4.3.2.1 Starch

Starch is one of the storage polysaccharides present in plants. All important food plants produce seeds containing starch as the carbohydrate reserve which serves as the principal food stored for use by the embryonic part in the initial growth stages. Starch is composed of two closely related polysaccharides amylase and amylopectin. Seed starches are classified either as cereal starch or tuber starch depending upon attaining power and physical properties. In oilseeds, starch is the main storage carbohydrate, as sugars are present in lesser amounts. The starch content of linseed 24.82 %.

4.3.2.2 Total sugars

Most important disaccharides occurring in plant food are sucrose and melibiose. The oligosaccharides are reducing and non-reducing. The total sugar content of three linseed was found out to be 0.406 per cent.

4.3.2.3 Reducing sugars

The reducing sugar content of linseed was 0.333 %.

4.3.2.4 Non-reducing sugars

The non reducing sugar content was 0.073 %.

4.3.2.5 Energy

The calorific value of linseed was calculated out to be 528.05 kcal/100g.

Table 4.3 Estimation of available carbohydrates of linseed *(Linum usitatissimum L.).*

S.No.	Available carbohydrates	Results
1	Total Sugars(%)	0.406
2	Reducing Sugars (%)	0.333
3	Non-reducing Sugars (%)	0.073
4	Starch(%)	24.82
5	Energy content (kcal/100g)	528.05

4.3.3 Unavailable carbohydrates

Dietary fibre occurs as structural material in the cell wall of plant. It includes NDF, ADF, lignin, hemicellulose, cellulose, pectin substances, gums and mucilages. Total dietary fibre is defined as those polysaccharides except starch that are not available for digestion by gastrointestinal enzymes in human system.

4.3.3.1 Acid detergent fibres (ADF)

However, dietary fibre is subjected to some degradation by bacterial fermentation in the large intestine. ADF is a part of the fibre which is soluble in dilute acids and is one of the component of dietary fibre constituents. The ADF content of linseed was 31.07 per cent.

4.3.3.2 Neutral detergent fibres (NDF)

The NDF content of linseed was in 51.35 %.

4.3.3.3 Cellulose

Cellulose is a polysaccharides made up of repeating units of glucose with ß 1-6 linkages. Cellulose is not digested by digestive enzymes present in human system, but by cellulase produced by some moulds in ruminants. The cellulose content of linseed was approximated to be 13.96 per cent.

4.3.3.4 Hemicellulose

Hemi cellulose are the polysaccharides containing pentoses, hexoses and uronic acids. They are hydrolysed by hot dilute acids, but are not acted upon by the digestive juices in the human intestinal tract. The hemicelluloses was 20.26 per cent.

4.3.3.5 Lignin

Lignin is a part of the plant cell and contribute to the structural rigidity of plants. It is thought to be responsible for the resistance of cell wall to

microbial degradation. The lignin content of linseed was found out to be 14.64 per cent.

Table 4.4 The unavailable carbohydrates of linseed (*Linum usitatissimum L.*)

S.No.	Unavailable carbohydrates	Results
1	ADF(%)	31.07
2	NDF(%)	51.35
3	Cellulose(%)	16.44
4	Hemicellulose(%)	20.26
5	Lignin(%)	14.64

4.3.4 Protein content

4.3.4.1 True protein

True protein is determined by subtracting NPN (Non-protein nitrogen) from crude protein nitrogen, and then multiplied by factor 6.25.

The crude protein content was 18.37 per cent in linseed.

4.3.4.2 Non-protein nitrogen

Non-protein nitrogen present in food products is the nitrogen contents which does not contributes towards proteins in the body. The non-protein nitrogen content was estimated to be 0.11 per cent.

Table 4.5 The various protein contents of linseed (*Linum usitatissimum L.*)

S.No.	Protein contents	Results
1	Crude protein (%)	19.06
2	Non Protein Nitrogen (%)	0.11
3	True Protein (%)	18.37

4.3.5 Minerals

The minerals are inorganic elements that serve as a constituent of skeletal structures, regulating acid base equilibrium and as a component of an activator of enzymes. The body contains about 24 macro and micro minerals, all of which must be provided by the diet. Bones and teeth are made up of mainly calcium, magnesium and phosphorous.. There are certain trace elements which are also of immense importance like copper, zinc and iron. Iron is an important constituent of blood. In the present study, an effort has been made to estimate calcium, phosphorous, potassium, magnesium, sodium, zinc, copper and iron in linseed. The various minerals found in linseed was estimated and the observation are reported (**Table 4.6**).

Table 4.6 Estimation of minerals in linseed *(Linum usitatissimum L.)*

S.No.	Minerals	Results (mg/100g)
1	Calcium	174.80
2	Phosphorous	399.82
3	Potassium	1077.4
4	Magnesium	482.6
5	Sodium	24.99
6	Zinc	5.09
7	Copper	2.09
8	Iron	2.98

4.3.6 Anti nutritional factors

4.3.6.1 Phytic acid

Phytic acid is one of the ant-nutritional factors present in plant foods. It affects the digestion and absorption of minerals, so in the present study an attempt was made to analyse the phytic acid content of linseed. The study revealed that the phytic acid content of linseed was 1020 mg/100g.

4.3.6.2 Phytate phosphorus

Phytate phosphorous is the amount of phosphorus which is in bound form with phytic acid. This was found out to be 287.40 mg/100g.

Table 4.7 The anti nutritional factors of linseed (*Linum usitatissimum L.)*

S.No.	Anti nutritional factors	Result (mg/100g)
1	Phytic acid	1020.00
2	Phytate phosphorus	287.40

4.4 Product formulations and their consumers' acceptability

Linseed was incorporated at three levels 5,7, and 9 per cent in various traditional products. The traditional food formulation in which incorporation was done were,- *laddoo*, *chutney*, *roti*, *mathri*, *dhokla* and pan cake (*cheela*). These food formulations were chosen with the presumption that they are commonly prepared in a typical Indian house hold and that almost all the subjects chosen are familiar with these food items. A 10 member panel was chosen to judge the products for their consumer acceptability. The overall acceptability was understood with the help of a score card. Hedonic rating test is used to measure the consumer acceptability of the products on the scale of 9 points, ranging from 'extremely liked' a score of 9 to 'disliked extremely' a score of 0.

4.4.1 Nutritional composition of formulated products

Linseed was incorporated at 5, 7, and 9% level to the six food formulation viz. *ladoo,* pancake *(cheela), mathri, roti, dhokla, chutney.* The nutritional attributes viz. calorie, protein, fats, fibre and minerals of all the six traditional food formulations were calculated. It was found that in all the six formulations calorie content was increased remarkably with increase in level of incorporation of linseed. This was because of the high calorie content of the linseed. The protein content was also increased in all the food formulations with increase in level of incorporation of linseed except for *dhokla* and pancake (*cheela*) where protein content was almost the same in all level of incorporation and control. This is because of the fact that bengal gram flour was used as the major ingredient, which has a protein content almost same as that of linseed. Fat content was increased remarkably with incorporation of linseed in all the food formulations, as linseed contains high amount of fat. The fibre content was slightly increased with the increase in level of incorporation of linseed in all the food formulations, except pan cake (*cheela*) where fibre content was decreased to a small extent. The increase in mineral content was negligible for all the six food formulations when linseed was incorporated. The nutritional

composition of all the six food formulations are reported in follwoing tables.

Table 4.8 Effect of incorporation of linseed on the proximate composition attributes of *ladoo*

Incorporation / Attributes	Control	5%	7%	9%
Calorie (kcal)	420.6	430.05	433.83	437.61
Protein (g)	12.12	12.52	12.7	12.87
Fat (g)	1.7	3.21	3.82	4.43
Fibre (g)	1.9	2.04	2.09	2.16
Minerals (g)	2.7	2.77	2.8	2.83

Table 4.9 Effect of incorporation of linseed on the proximate composition attributes of pan cake (*cheela*)

Incorporation / Attributes	Control	5%	7%	9%
Calorie (kcal)	372	379.9	383	386.1
Protein (g)	20.8	19.76	19.34	18.92
Fat (g)	5.6	5.32	5.2	5.09
Fibre(g)	1.2	1.14	1.11	1.09
Minerals (g)	2.7	2.77	2.8	2.83

Table 4.10 Effect of incorporation of linseed on the proximate composition attributes of *mathri*

Incorporation / Attributes	control	5%	7%	9%
Calorie (kcal)	611	620.4	624.23	628
Protein (g)	12.1	12.5	12.68	12.85
Fat(g)	31.7	33.21	33.82	34.43
Fibre(g)	1.9	2.04	2.09	2.16
Minerals (g)	2.7	2.77	2.8	2.83

Table 4.11 Effect of incorporation of linseed on the proximate composition attributes of *roti*

Incorporation / Attributes	control	5%	7%	9%
Calorie (kcal)	341	350.4	354.23	358
Protein (g)	12.1	12.50	12.68	12.85
Fat(g)	1.7	3.21	3.82	4.43
Fibre(g)	1.9	2.04	2.09	2.16
Minerals (g)	2.7	2.77	2.8	2.83

Table 4.12 Effect of incorporation of linseed on the proximate composition attributes of *chutney*

Incorporation / Attributes	control	5%	7%	9%
Calorie (kcal)	221.7	246	255.72	265.44
Protein (g)	4.22	5.06	5.41	5.76
Fat(g)	2.96	4.53	5.15	5.78
Fibre(g)	5.31	5.49	5.56	5.63
Minerals (g)	3.89	3.98	4.01	4.05

Table 4.13 Effect of incorporation of linseed on the proximate composition attributes of *dhokla*

Incorporation / Attributes	control	5%	7%	9%
Calorie (kcal)	391.9	399.8	402.9	406.1
Protein (g)	20.8	20.77	20.77	20.76
Fat(g)	5.6	6.93	7.44	7.97
Fibre(g)	1.2	1.38	1.44	1.52
Minerals (g)	2.7	2.77	2.8	2.83

4.4.2 Selection of a product

This exercise was carried out to choose the most liked product, based on its Hedonic Rating Score, from the six food formulations prepared for the study. The sensory evaluation by 9 point hedonic scale revealed the consumers' acceptability for different products **(Table 4.14)**. The value of the scores exhibited in the table for a particular food formulation is the mean value of the scores as calculated from the scores given by the

Table 4.14 Hedonic rating scores for the different food formulations

Incorporation level of linseed / Food formulation	0% (control)	5%	7%	9%
Roti	7.8	7.6	7.5	5.9
Mathri	8.0	7.8	7.6	7.3
Pancake	7.5	6.7	6.0	5.4
Chutney	7.0	6.2	5.5	5.0
Dhokla	7.1	6.8	6.1	5.6
Ladoo	8.2	8.4	8.0	7.2

panel of 10 sensory evaluators. Results indicate that in the control (0% incorporation of linseed), *ladoo* was the most preferred food formulation followed by *mathri*, *roti* , pan cake, *dhokla* and *chutney*, respectively. In the 5% and 7% incorporation levels, the order of

preference was almost the same as in control viz. ladoo was the most preferred food formulation. In 9% incorporation level there were some changes in the order of preference then the other two incorporation levels; *mathri* was the most preferred followed by *ladoo* and *roti*, *dhokla.* pancake and *chutney* followed in that order. The comparative mean Hedonic Rating Scores for the different linseed incorporated food formulations is as shown in **Fig 4.1**. The most preferred food formulation, which shall be served to the subjects, based on the Hedonic Rating Scores is, - *ladoo*.

4.4.3 Selection of level of incorporation of linseed

It was imperative that incorporation of linseed would result in some change in the taste and other organoleptic characteristics of the food formulations. This essentiated in carrying out some statistical test to learn the effect of significance of incorporating linseed to the overall consumers' acceptability of linseed. A statistical tool, paired t-test **(Table 4.15)** was used to study significance of the effect of incorporation of linseed in the overall acceptability of the food formulations. The null hypothesis (H_0) was defined as, there is no significant difference in the means of Hedonic Rating Scores for the control (0% incorporation of linseed) and for the linseed incorporated food formulation (5%, 7%, and 9%,). The alternate hypothesis (H_1) was

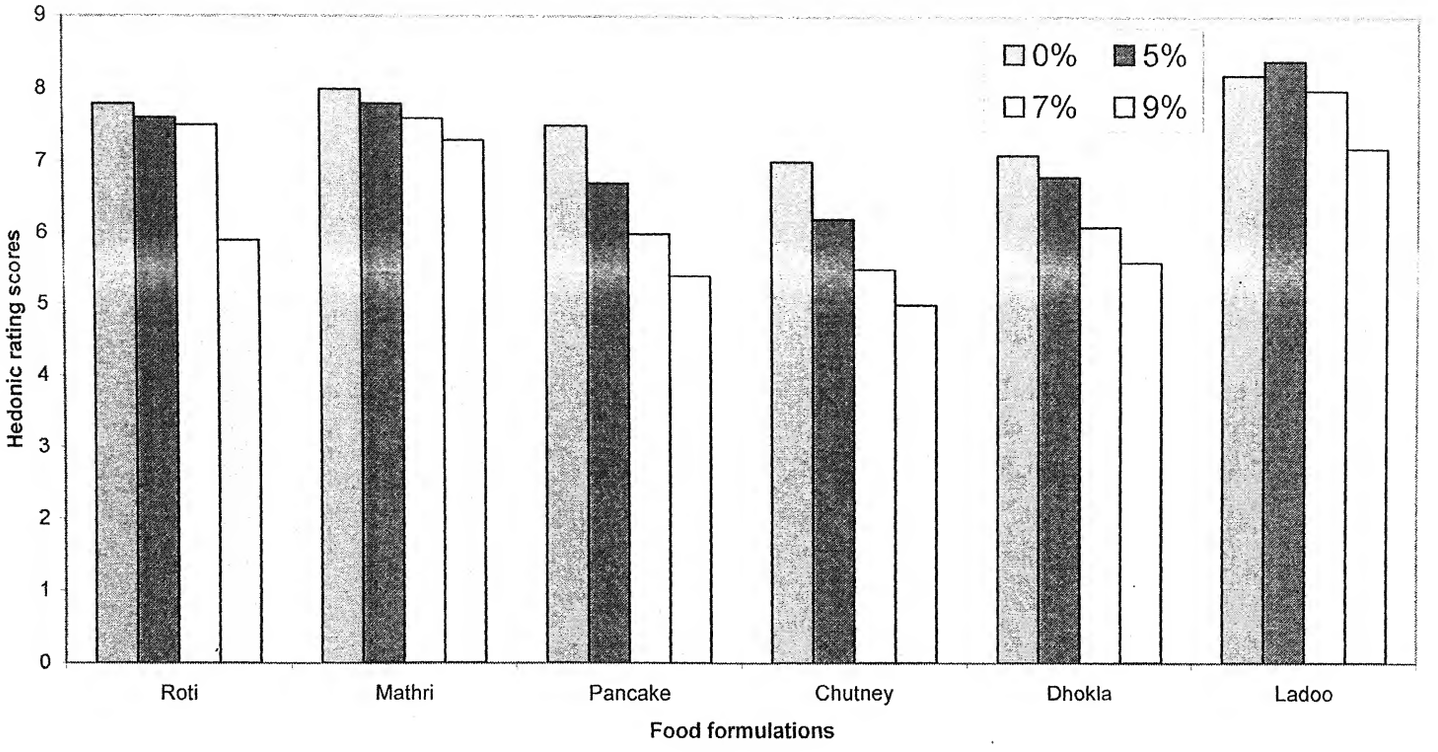

Fig. 4.1 Comparative mean Hedonic rating scores for the different linseed incorporated food formulations

defined as; there is significance difference between both the means. The results of the paired t – test indicate that the H_0 is accepted for 5 and for 7% levels ($t_{cal} < t_{(0.05.6)}$) while it is rejected at 9 % level ($t_{cal} > t_{(0.05,6)}$) **(Table 4.15)**. This establishes that linseed can be incorporated at either 5 or 7% level with out making any significant difference to the overall consumer acceptability. Since the research work aims at maximizing the hypolipidemic effect of linseed so 7% was chosen to be the level of incorporation in the traditional food product.

Table 4.15 t-test analysis for selection of level of incorporation

t-test Incorporation levels	Standard error (S.E.)	t_{cal}	$t_{(0.05,6)}$	Hypothesis status
5%	0.2167	1.624	1.943	$t_{cal} < t_{(0.05,6)}$: H_0 accepted
7%	0.4397	1.865	1.943	$t_{cal} < t_{(0.05.6)}$: H_0 accepted
9%	0.5281	2.594	1.943	$t_{cal} > t_{(0.05,6)}$: H_0 rejected

4.5 Effect of administering linseed to the hyperlipidemic subjects

Hyperlipidemia means a rise in the level of blood lipids (cholesterol and triglycerides) and lipoproteins (LDL, VLDL etc.) form their normal values. As per the objective of the study it was desired to bring the raised blood lipids and lipoproteins to their normal values. Linseed was incorporated at 7% level in a traditional food formulation,- *ladoo* and

was inculcated in the diet of 50 hyperlipidemic subjects daily for six weeks, which means that the amount of linseed consumed was 7 g per day. It was proposed that suitable adjustment in the diet pattern be made so as to accommodate the calorie as provided by the linseed-incorporated *ladoo*. The effect of feeding linseed was evaluated by monitoring the blood samples of the 50 hyperlipidemic subjects for,- serum total cholesterol content, HDL cholesterol content, serum triglycerides content, VLDL cholesterol content, LDL cholesterol content, and LDL: HDL content.

4.5.1 Effect of administering linseed on serum total cholesterol content

Cholesterol is present in all animal tissues but is usually absent from food of plant origin. But sterols are present in the plants, which re called as phytosterols. Cholesterol plays three important role in the human body. It is a structural component of all cell membrane, it also is a precursor of bile acids, it is also a precursor to adrenal and gonadal hormone and vitamin D. But presence of choloesterol in the blood is also a major cause of death of human beings across the globe. An increase in the serum total cholesterol is known to cause changes in arterial structure ultimately leading to atherosclerosis **(Stamler J.1978)** and **(Gernity *et al.* 1961)**. In the present study efforts were made to reduce the serum total cholesterol of the fifty hyperlipidemic subjects by

serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The results show **(Table 4.16)** that when the subjects were administered linseed the serum total cholesterol decreased significantly ($t_{cal} > t_{0.05,49}$). The percentage decrease in the serum total cholesterol was found to be 7.10%. The results also show **(Table 4.17)** that when the subjects were not administered linseed the serum total cholesterol was not significantly ($t_{cal} < t_{0.05,49}$) affected. The percentage change in the serum total cholesterol was also negligible. The blood cholesterol lowering action of linseed might be due to the presence of alpha-linolenic acid (ALA), a polyunsaturated fatty acid which constitutes about 57 per cent of the total fatty acids in linseed. **Kolodziejczyk *et al.* (1995)** reported that linseed oil is rich in ω-3 fatty acids known to influence blood platelet aggregation, lowering blood cholesterol concentration and prevents coronary heart disease. **Kritchevsky *et al.* (1991)** observed a fall in blood cholesterol level of rats given higher amount of linseed. **Auer (1991)** observed a decrease in serum cholesterol level. According to him, serum cholesterol level decreased from 205 mg/100 to 157 mg/ 100 ml in 5 per cent linseed group. **Bierenbaum *et al.* (1993)** observed a 7 per cent decrease in blood total cholesterol in a

Table 4.16 t-test analysis of serum total cholesterol content at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average serum total cholesterol level (mg/100ml of blood)	$\overline{x}$	287.04
2	final average serum total cholesterol level (mg/100ml of blood)	$\overline{y}$	266.64
3	change in average serum total cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	20.39
4	percent change	δ	7.10
5	standard error	S.E.	3.04
6	t-test value calculated	$\lvert t_{cal}\rvert$	6.685
7	t-test value table	$\lvert t_{0.05,49}\rvert$	2.5

Table 4.17 t-test analysis of serum total cholesterol content at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average serum total cholesterol level(mg/100ml of blood)	$\overline{x}$	287.04
2	final average serum total cholesterol level (mg/100ml of blood)	$\overline{y}$	287.02
3	change in average serum total cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	0.02
4	percent change	δ	0
5	standard error	S.E.	0.073
6	t-test value calculated	$\lvert t_{cal} \rvert$	0.286
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

group of 15 hyperlipidemic men and women who were fed on diet containing 15 g linseed. The changes in serum total cholesterol content at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

4.5.3 Effect of administering linseed on HDL cholesterol content

The high density lipoprotein (HDL) cholesterol contains less lipid and more protein hence the density is very high. It is also synthesized in the liver from endogenous fat sources. HDL transfers cholesterol from the tissues to the liver for catabolism and excretion, higher levels of serum HDL are considered protective against cardiovascular disease. In the present study efforts were made to increase the high density lipoprotein (HDL) cholesterol of the fifty hyperlipidemic subjects by serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The results show **(Table 4.18)** that when the subjects were administered linseed the high density lipoprotein (HDL) cholesterol increase significantly ($t_{cal} > t_{0.05,49}$). The percentage increase in the high density lipoprotein (HDL) cholesterol was found to be 10.33%. The results also show **(Table 4.19)** that when the subjects were not administered linseed the high density lipoprotein (HDL) cholesterol was not significantly ($t_{cal} < t_{0.05,49}$)

Table 4.18 t-test analysis of HDL cholesterol content at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average HDL cholesterol level (mg/100ml of blood)	$\overline{x}$	47.13
2	final average HDL cholesterol level (mg/100ml of blood)	$\overline{y}$	52.01
3	change in average HDL cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	4.87
4	percent change	δ	10.33
5	standard error	S.E.	0.820
6	t-test value calculated	$\lvert t_{cal} \rvert$	5.939
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

Table 4.19 t-test analysis of HDL cholesterol content at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average HDL cholesterol level (mg/100ml of blood)	$\overline{x}$	47.13
2	final average HDL cholesterol level (mg/100ml of blood)	$\overline{y}$	47.13
3	change in average HDL cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	0.0
4	percent change	δ	0
5	standard error	S.E.	0.076
6	t-test value calculated	$\lvert t_{cal} \rvert$	0.01
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

affected. The percentage change in the high density lipoprotein (HDL) cholesterol was also negligible. **Dubey and Thakur (1979)** reported that incorporation of linseed oil in diet reduces atherosclerosis in rabbits. The changes in high-density lipoprotein (HDL) cholesterol content at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

4.5.4 Effect of administering linseed on serum triglycerides content

Triglycerides are made up of two major components glycerol and fatty acids. The glycerol portion is common to almost all dietary fats. The fatty acid component which is attached to one or two or three of the hydroxy groups in glycerol, varies widely in composition from one fat to another. In the present study efforts were made to reduce the serum triglycerides of the fifty hyperlipidemic subjects by serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The results show **(Table 4.20)** that when the subjects were administered linseed the serum triglycerides decreases significantly ($t_{cal} > t_{0.05,49}$). The percentage decrease in the serum triglycerides was found to be 6.34 %. The results also show **(Table 4.21)** that when the subjects were not administered linseed the serum triglycerides was not significantly ($t_{cal} < t_{0.05,49}$) affected. **Bierenbaum *et al.* (1993)** reported that blood

Table 4.20t-test analysis of serum triglycerides content at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average serum triglycerides level (mg/100ml of blood)	$\overline{x}$	206.61
2	final average serum triglycerides level (mg/100ml of blood)	$\overline{y}$	193.51
3	change in average serum triglycerides level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	13.1
4	percent change	δ	6.34
5	standard error	S.E.	3.0155
6	t-test value calculated	$\lvert t_{cal}\rvert$	4.344
7	t-test value table	$\lvert t_{0.05,49}\rvert$	2.5

Table 4.21 t-test analysis of serum triglycerides content at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average serum triglycerides level (mg/100ml of blood)	$\overline{x}$	206.50
2	final average serum triglycerides level (mg/100ml of blood)	$\overline{y}$	206.61
3	change in average serum triglycerides level (mg/100ml of blood)	$\lvert \overline{y} - \overline{x} \rvert$	0.063
4	percent change	δ	0
5	standard error	S.E.	0.0678
6	t-test value calculated	$\lvert t_{cal} \rvert$	0.93
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

triglycerides decreases slightly but not significantly during flax seed consumption. **Arora** and **Modgil (2000)** reported a significant ($p<0.05$) decrease is in blood triglycerides level of rats fed on linseed. The changes in serum triglycerides content at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

4.5.5 Effect of administering linseed on VLDL cholesterol content

The very high-density lipoproteins (VLDL) are large lipids primarily comprising triglycerides and contain about 10 to 15% cholesterol. The VLDL are synthesized in the liver from endogenous sources and transports endogenous triglycerides to the cells. In the present study efforts were made to reduce the serum triglycerides of the fifty hyperlipidemic subjects by serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The results show **(Table 4.22)** that when the subjects were administered linseed the very high density lipoproteins (VLDL) decreases significantly ($t_{cal} > t_{0.05,49}$). The percentage decrease in the very high density lipoproteins (VLDL) was found to be 6.46 %. The results also show **(Table 4.23)** that when the subjects were not administered linseed the serum triglycerides was not significantly ($t_{cal} < t_{0.05,49}$) affected. The changes in very high-density

Table 4.22 t-test analysis of VLDL cholesterol content at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average VLDL cholesterol level (mg/100ml of blood)	$\overline{x}$	41.32
2	final average VLDL cholesterol level (mg/100ml of blood)	$\overline{y}$	38.70
3	change in average VLDL cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	2.67
4	percent change	δ	6.46
5	standard error	S.E.	0.610
6	t-test value calculated	$\lvert t_{cal}\rvert$	4.377
7	t-test value table	$\lvert t_{0.05,49}\rvert$	2.5

Table 4.23 t-test analysis of VLDL cholesterol content at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average VLDL cholesterol level (mg/100ml of blood)	$\overline{x}$	41.31
2	final average VLDL cholesterol level (mg/100ml of blood)	$\overline{y}$	41.32
3	change in average VLDL cholesterol level (mg/100ml of blood)	$\lvert \overline{y} - \overline{x} \rvert$	0.013
4	percent change	δ	0
5	standard error	S.E.	0.0135
6	t-test value calculated	$\lvert t_{cal} \rvert$	0.963
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

lipoproteins (VLDL) content at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

4.5.6 Effect of administering linseed on LDL cholesterol content

In the present study efforts were made to reduce the low density lipoproteins (LDL) cholesterol of the fifty hyperlipidemic subjects by serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The results show **(Table 4.24)** that when the subjects were administered linseed the low-density lipoproteins (LDL) cholesterol decreases significantly ($t_{cal} > t_{0.05,49}$). The percentage decrease in the low density lipoproteins (LDL) cholesterol was found to be 11.40 %. The results also show **(Table 4.25)** that when the subjects were not administered linseed the low density lipoproteins (LDL) cholesterol was not significantly ($t_{cal} < t_{0.05,49}$) affected. **Bierenbaum *et al.* (1993)** reported that LDL cholesterol values were reduced by 11 per cent during flaxseed consumption. **Cunnane *et al.* (1993)** observed 18 per cent decrease in LDL cholesterol when healthy women consumed linseed daily for 4 weeks. **Arora** and **Modgil (2000)** reported a significant ($p<0.05$) decrease is in LDL cholesterol level of rats fed on linseed. The change in LDL cholesterol content at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

Table 4.24 t-test analysis of LDL cholesterol content at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average LDL cholesterol level (mg/100ml of blood)	$\overline{x}$	198.58
2	final average LDL cholesterol level (mg/100ml of blood)	$\overline{y}$	175.93
3	change in average LDL cholesterol level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	22.64
4	percent change	δ	11.40
5	standard error	S.E.	3.401
6	t-test value calculated	$\lvert t_{cal}\rvert$	6.658
7	t-test value table	$\lvert t_{0.05.49}\rvert$	2.5

Table 4.25 t-test analysis of LDL cholesterol content at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average LDL cholesterol level (mg/100ml of blood)	$\overline{x}$	198.51
2	final average LDL cholesterol level (mg/100ml of blood)	$\overline{y}$	198.58
3	change in average LDL cholesterol level (mg/100ml of blood)	$\lvert \overline{y} - \overline{x} \rvert$	0.072
4	percent change	δ	0
5	standard error	S.E.	0.068
6	t-test value calculated	$\lvert t_{cal} \rvert$	1.058
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

4.5.7 Effect of administering linseed on LDL: HDL cholesterol

In the present study efforts were made to study the ratio of low-density lipoproteins cholesterol and high density lipoproteins cholesterol (LDL: HDL) of the fifty hyperlipidemic subjects by serving linseed incorporated at 7% of the major ingredient, daily for a period of six weeks, in the form of traditional food formulation, *ladoo*. The study of the ratio of low-density lipoproteins cholesterol and high density lipoproteins cholesterol (LDL: HDL) was important as the risk of cardio vascular diseases is directly proportional to the LDL: HDL ratio. Lower is the LDL: HDL ratio lesser is the chance of acquiring cardio vascular disease. The results show **(Table 4.26)** that when the subjects were administered linseed the ratio of low-density lipoproteins cholesterol and high density lipoproteins cholesterol (LDL: HDL) decreases significantly ($t_{cal} > t_{0.05,49}$). The percentage decrease in the ratio of low density lipoproteins cholesterol and high density lipoproteins cholesterol (LDL : HDL) was found to be almost21%. The results also show **(Table 4.27)** that when the subjects were not administered linseed the low-density lipoproteins cholesterol and high-density lipoproteins cholesterol (LDL: HDL) was not significantly ($t_{cal} < t_{0.05,49}$) affected. The decrease in the ratio of low-density lipoproteins cholesterol and high-density lipoproteins cholesterol (LDL: HDL) was because of the fact that, after administering linseed to the fifty hyperlipidemic subjects the

Table 4.26 t-test analysis of LDL: HDL cholesterol at experimental conditions

S.No.	Statistical tools	Notations	Values
1	initial average LDL: HDL level (mg/100ml of blood)	$\overline{x}$	4.488
2	final average LDL: HDL level (mg/100ml of blood)	$\overline{y}$	3.543
3	change in average LDL: HDL level (mg/100ml of blood)	$\lvert \overline{y} - \overline{x} \rvert$	0.945
4	percent change	δ	21.05
5	standard error	S.E.	0.1552
6	t-test value calculated	$\lvert t_{cal} \rvert$	6.089
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

Table 4.27 t-test analysis of LDL: HDL cholesterol at control conditions

S.No.	Statistical tools	Notations	Values
1	initial average LDL: HDL level (mg/100ml of blood)	$\overline{x}$	4.21
2	final average LDL: HDL level (mg/100ml of blood)	$\overline{y}$	4.88
3	change in average LDL: HDL level (mg/100ml of blood)	$\lvert\overline{y} - \overline{x}\rvert$	0.002
4	percent change	δ	0
5	standard error	S.E.	0.0089
6	t-test value calculated	$\lvert t_{cal} \rvert$	0.225
7	t-test value table	$\lvert t_{0.05,49} \rvert$	2.5

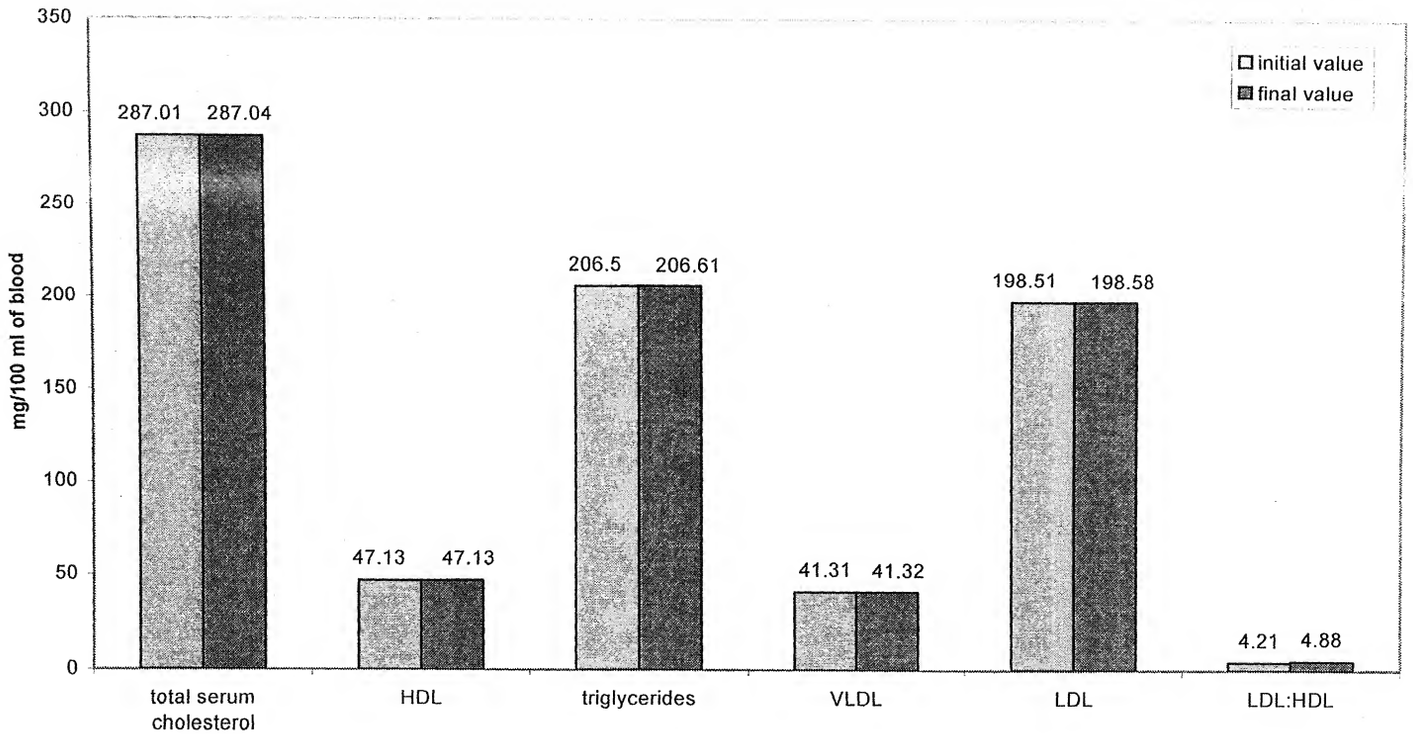

Fig 4.2 Change in blood lipid profile at control conditions

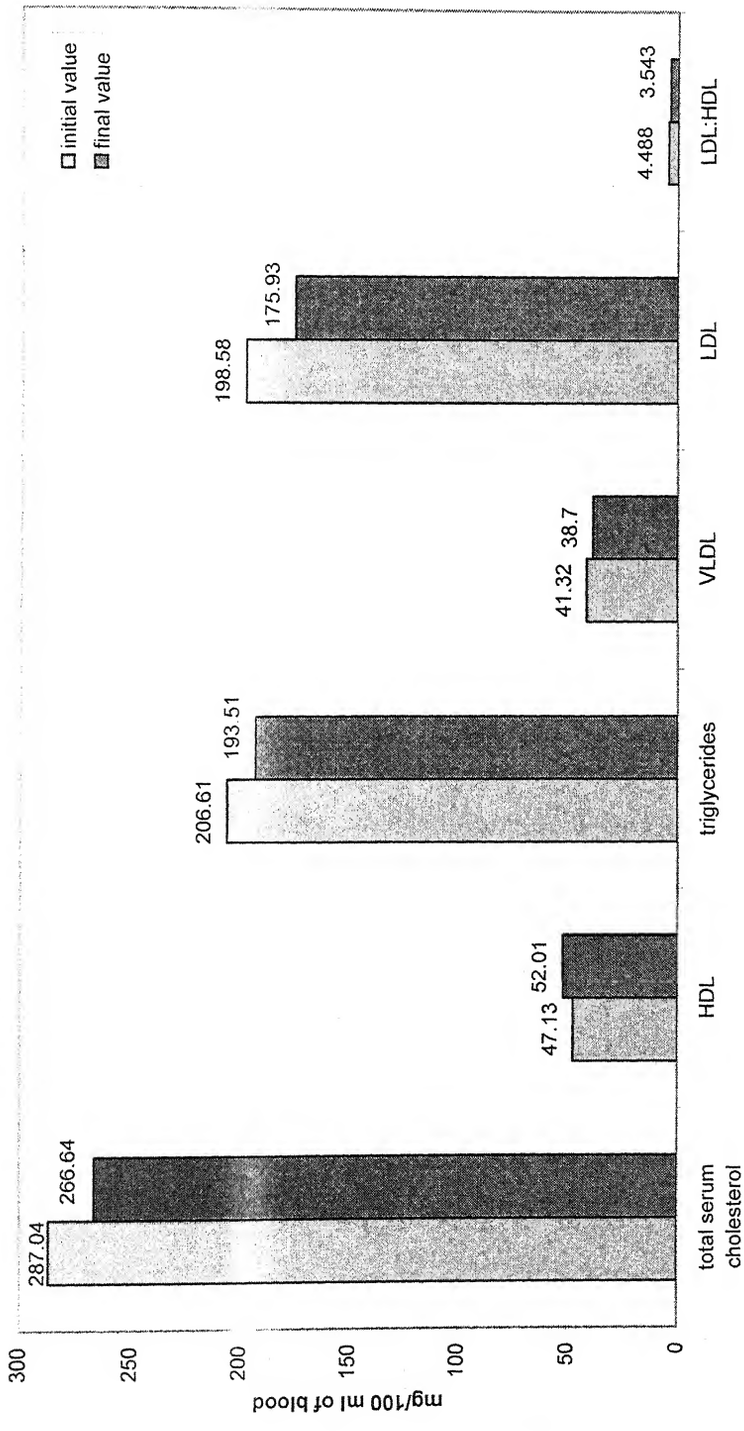

Fig 4.3 Change in blood lipid profile in at experimental conditions

high-density lipoproteins cholesterol (HDL) content of their blood increased significantly while the low-density lipoproteins cholesterol (LDL) content decreased significantly. **Arora** and **Modgil (2000)** reported that the low-density lipoproteins cholesterol and high-density lipoproteins cholesterol (LDL: HDL) was decreased after consumption of linseed. **Ratnayake *et al.* (1992)** also reported lowering in the LDL: HDL cholesterol when linseed was fed to the rats. The change in LDL: HDL at experimental and control conditions have been depicted in **Fig. 4.2** and in **Fig. 4.3**.

SUMMARY

AND

CONCLUSIONS

Chapter-V

SUMMARY AND CONCLUSIONS

The present research work was carried out to a comprehensive physico chemical analysis of linseed and to study the hypocholesterolemic effect of linseed as and when it is inculcated in the diet of cardio vascular patients.

- A detailed study of all the physical and chemical analysis of linseed was done. It was found that the linseed was shiny dark brown in colour, flat oval with slight pointed tip in shape. Average length and width of linseed was found to be 5.45 and 1.45 mm.
- The chemical analysis revealed moisture, ash, crude fat, crude protein and crude fibre, content to be 6.25, 4.17, 42.65, 19.06 and 8.05 per cent, respectively. In oilseeds starch is the main storage carbohydrate. The starch content of linseed was found to be 24.82 per cent where as total sugars were found to be 0.406 per cent. The reducing sugar content of linseed was found to be 0.388 per cent. The calorific value was found to be 528.03 kcal/100g. The unavailable carbohydrates found were ADF (31.07 per cent), NDF (51.35 per cent), cellulose (16.44 per cent), hemi cellulose (20.26 per cent) and lignin (14.64 per cent).The non protein nitrogen and true protein content was found to be 0.11 per cent and 18.37 per cent, respectively.

- Amongst the macro minerals, potassium was found to be maximum, 1077.40 mg/100gm and sodium was found to be minimum 24.99 mg/100gm. Other minerals present were phosphorus (399.82 mg/100gm), magnesium (482.60 mg/100gm), calcium (174.80 mg/100gm). Trace elements were also found in linseed viz. zinc (5.09mg/100gm), copper (2.09mg/100gm) and iron (2.98mg/100gm).
- It was also concluded that linseed contain some anti nutritional factors also, like- phytic acid (1020.0mg/100gm), phytate phosphorous (287.40 mg/100gm).
- Linseed was incorporated at 5 per cent, 7 per cent, and 9 per cent in six traditional food formulations namely,- *ladoo*, *roti*, pan cake (*cheela*), *dhokla*, *mathri*, and *chutney*. The nutritional attributes viz. calorie, protein, fats, fibre and minerals of all the six traditional food formulations were calculated. It was found that in all the six formulations calorie content was increased remarkably with increase in level of incorporation of linseed. This was because of the high calorie content of the linseed. The protein content was also increased in all the food formulations with increase in level of incorporation of linseed except for *dhokla* and pancake (*cheela*) where protein content was almost the same in all level of incorporation and control. This is because of the fact that bengal gram flour was used as the major

ingredient, which has a protein content almost same as that of linseed. Fat content was increased remarkably with incorporation of linseed in all the food formulations, as linseed contains high amount of fat. The fibre content was slightly increased with the increase in level of incorporation of linseed in all the food formulations, except pan cake (*cheela*) where fibre content was decreased to a small extent. The increase in mineral content was negligible for all the six food formulations when linseed was incorporated.

- In control conditions (0% linseed incorporation), *ladoo* was the most preferred fod formulation followed by *mathri*, *roti*, pan cake (*cheela*), *dhokla, chutney.* At 5 and 7 per cent incorporation levels, the order of preference was almost the same as in control viz. ladoo was the most preferred food formulation. In 9% incorporation level there were some changes in the order of preference of the food formulations. In this case *mathri* was the most preferred followed by *ladoo*, *roti*, *dhokla*, pan cake (*cheela*) and *chutney* in that order.
- On the basis of the sensory evaluation it was found that the food product containing linseed up to 9 per cent level were in acceptance range. But the acceptability of the sample containing 9 per cent linseed differed significantly with respect to the acceptance of the sample prepared under control conditions. While the difference in the

acceptance of the sample containing 5 and 7 per cent linseed with respect to the acceptance of the sample prepared under the control conditions was insignificant. The incorporation level was thus chosen to be 7%.

- Among the different food formulations *ladoo* was the most accepted as compared to the other food formulations, hence, *ladoo* having 7% linseed (i.e. 7 gm) was served to the cardiovascular patients daily for a period of six weeks. It was proposed that suitable adjustment in the diet pattern be made so as to accommodate the calorie as provided by the linseed incorporated *ladoo*.
- For the purpose of the research, 50 hyperlipidemic patients were chosen from the Jhansi city. The status of the patients being hyperlipidemic was judged on the basis of serum total cholesterol, low-density lipoprotein (LDL) cholesterol and serum triglycerides.
- While serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and serum triglycerides were estimated using standard analytical techniques in pathological laboratories, the low-density lipoprotein (LDL) and (VLDL) cholesterol, was estimated by using the **Friedewald (1972)** equation.
- It was hypothesized that serving linseed to the cardiovascular patients would significantly lower the blood lipids and lipoproteins.

- At first the blood lipid and lipo protein content of the subjects were recorded. The subjects were then allowed to continue their normal diet patterns. After a period of six weeks the blood lipids and lipoproteins content of the subjects were checked. It was found that there was no significant difference in the blood lipids and lipo protein content of the subjects. The significance was established statistically by paired t-test.
- The subjects were then served linseed incorporated to *ladoo* at 7% (i.e. 7 gm) daily for a period of six weeks.
- After the period of six weeks the blood lipids and lipo protein content of the subjects were recorded. It was found that serum total cholesterol was decreased by 7.10 per cent, low-density lipoprotein (LDL) cholesterol was decreased by 11.40 per cent, serum triglycerides was decreased by 6.34 per cent, very low-density lipoprotein (VLDL) cholesterol was decreased by 6.46 per cent, high-density lipoprotein (HDL) cholesterol was increased by 10.33 per cent, and LDL: HDL was decreased by 21.05 per cent. All these results were significant as was statistically established by paired t-test.

Therefore, it can be concluded form the above research work that linseed incorporated traditional food formulation *ladoo* can served to hyperlipidemic patients for significantly reducing the blood lipids and lipo proteins which

are responsible for cardiovascular diseases, namely- serum total cholesterol, low-density lipoprotein (LDL) cholesterol, serum triglycerides, very low-density lipoprotein (VLDL) cholesterol, LDL: HDL cholesterol and increasing high-density lipoprotein (HDL).

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